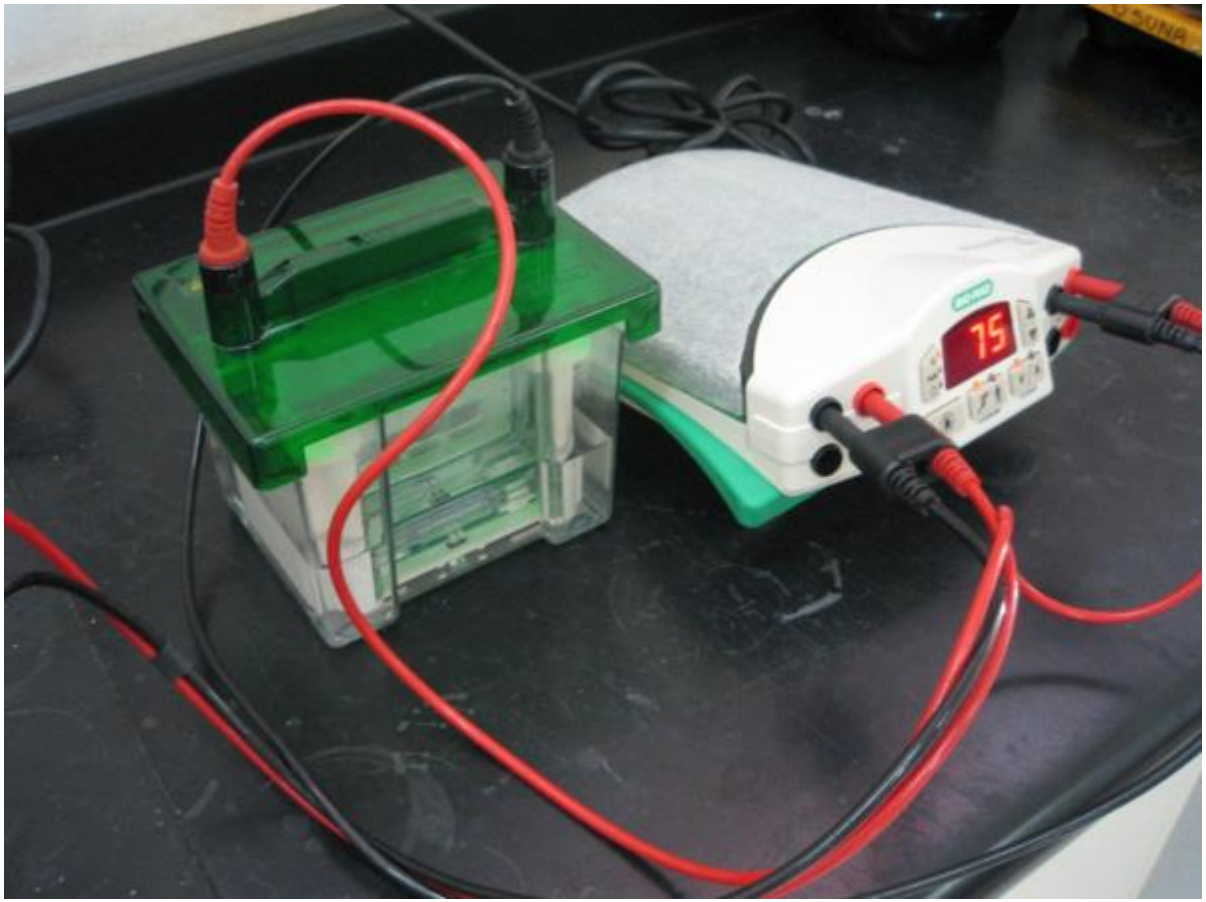
ELECTROFORESIS

jareig@umh.es



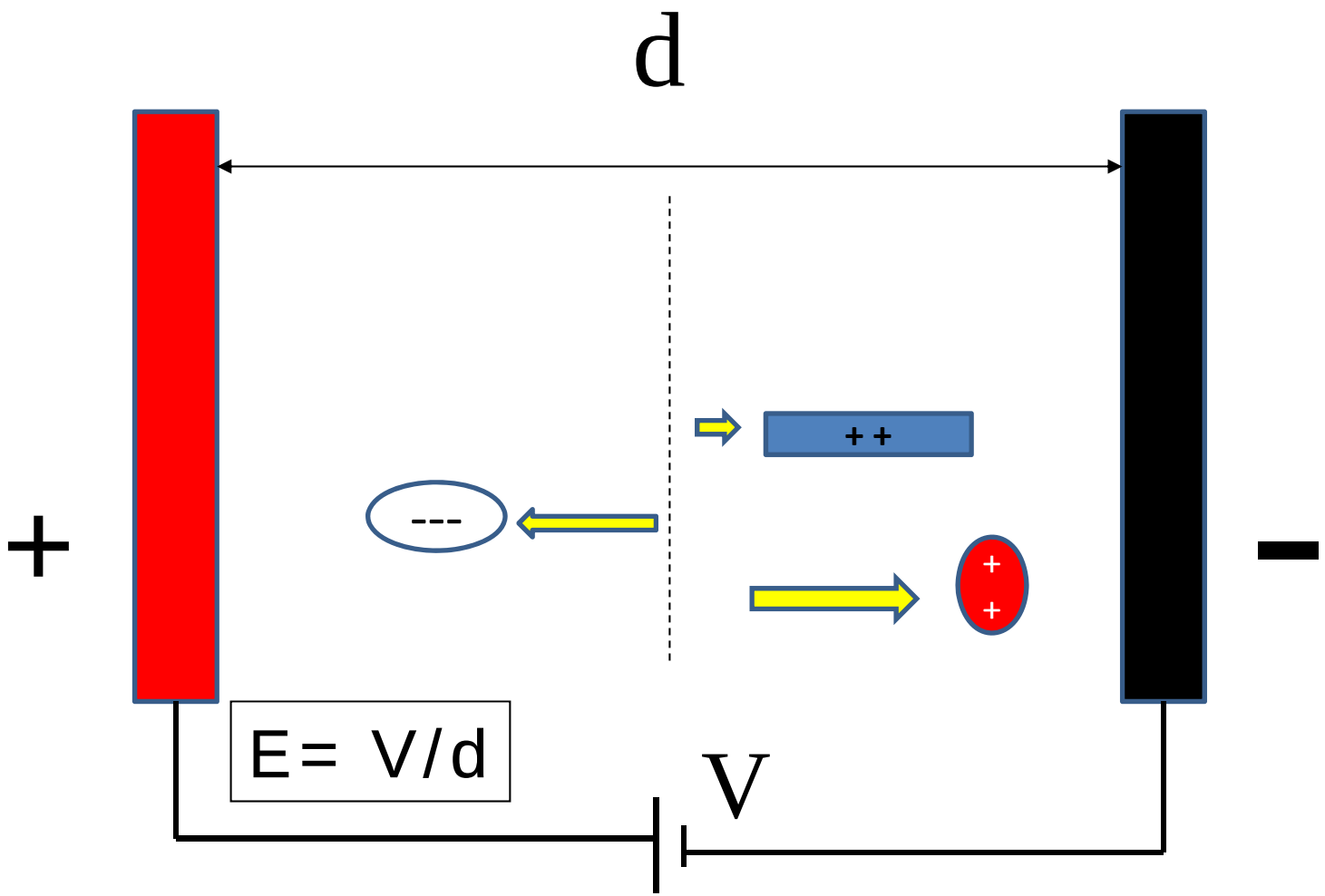
FUENTE
DE ALIMENTACIÓN

CUBETA

ELECTROFORESIS: TRANSPORTE DE MOLÉCULAS A TRAVÉS DE UN CAMPO ELECTRICO
TECNOLOGÍA ANALÍTICA O TECNOLOGIA PREPARATIVA➔PROTEÓMICA

LA CARGA ELECTRICA ESTÁ ASOCIADA FRECUENTEMENTE CON LAS BIOMOLÉCULAS

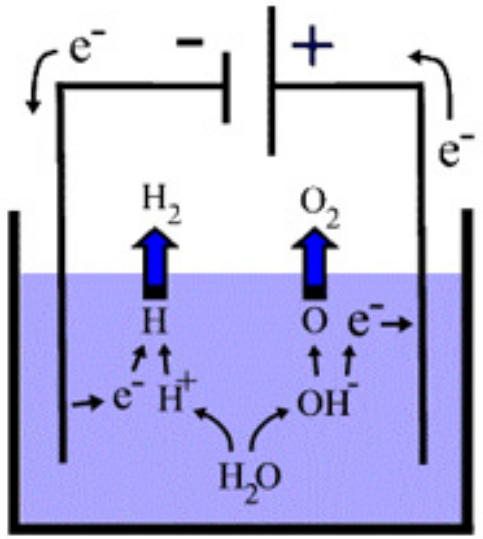
$-NH_3^+$ $-COO^-$ $-PO_3^-$



$\mu r = \phi$ (carga, tamaño, forma, viscosidad…)

ELECTROFORESIS ➔: ELECTROLISIS➔*"burbujas y calor"*
La corrriente entre electrodos se mantiene por los electrolitos del buffer que mantienen **pH constante**

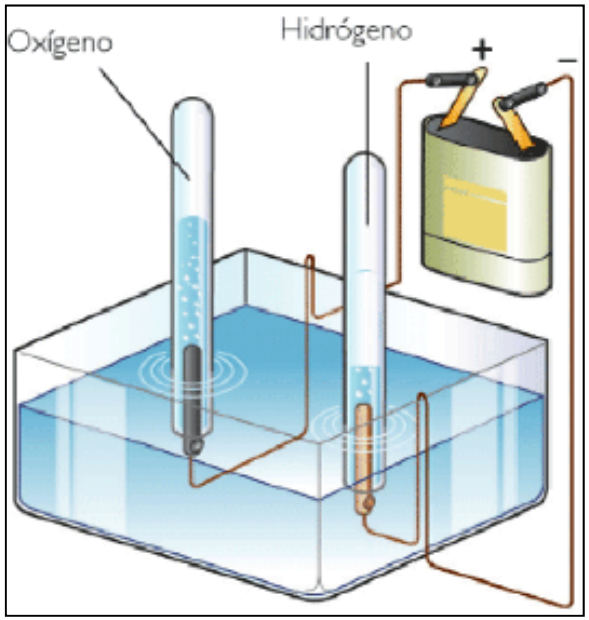
Electrolysis: Splitting water with electricity to produce hydrogen and oxygen:



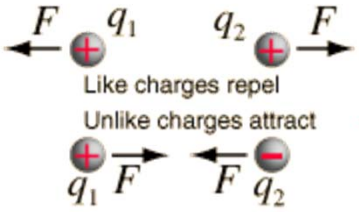
alcalinización acidificación

$V = R \times I$

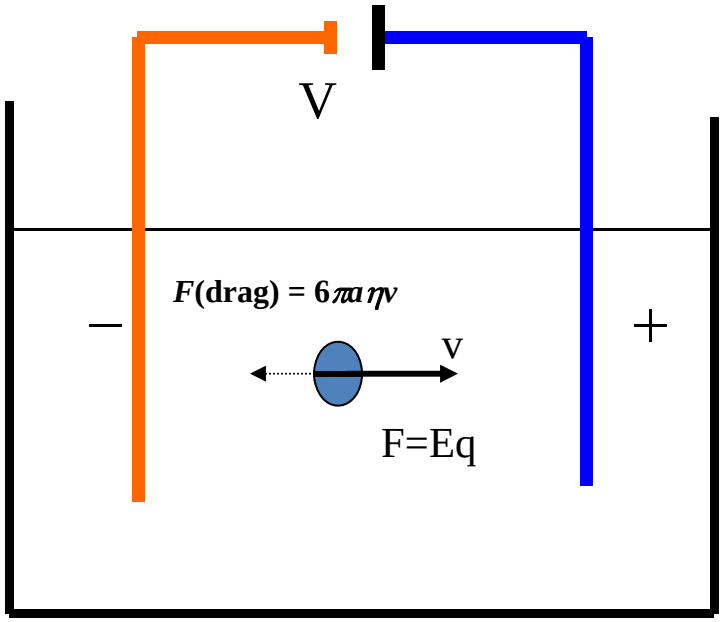
$W = I^2 \times R$



En polo negativo (CATODO) $H_2O + 2\ e^- \rightarrow OH^- + H_2$
En polo positivo (ANODO) $H_2O \rightarrow 2e^- + 2H^+ + 1/2O_2$



$$F = \frac{kq_1q_2}{r^2} = \frac{q_1q_2}{4\pi\varepsilon_0 r^2}$$ *Coulomb's Law*



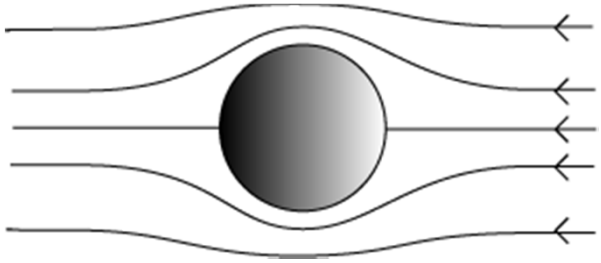
v=F/f

f; coeficiente friccional

v= Eq/f= Eq/6πηr

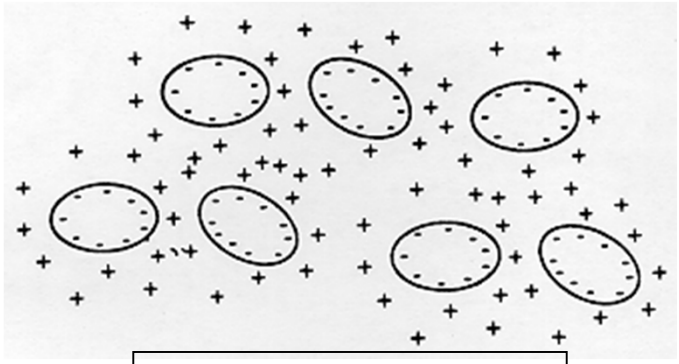
μ=v/E= q/6πηr

μ; Movilidad electroforética



Ley Stokes en condiciones ideales

ATMOSFERA IONICA TEORIA DEBYE-HUCKEL



Función de Henry

$$v = \frac{Eq/6\pi r\eta \times 1/1+KR}{\text{ideal x factor de corrección}}$$

$$K=[8\pi Ne^2/1000DkT]^{1/2} \times I^{\frac{1}{2}}$$

D=constante dieléctirca; I=fuerza iónica

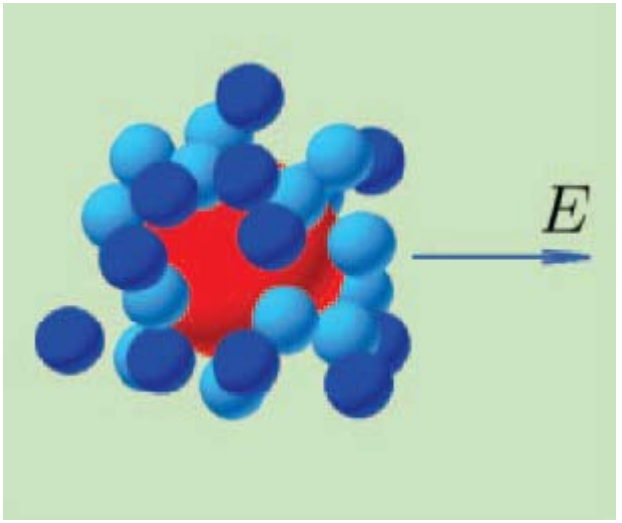
Bioquimica fisica, Van Holde ED Exedra

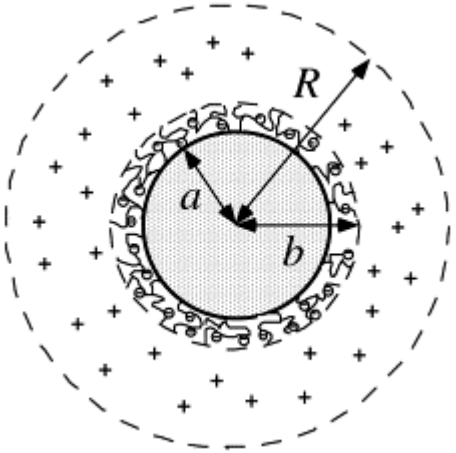
Caso ideal $v=Eq/6\pi r\eta$

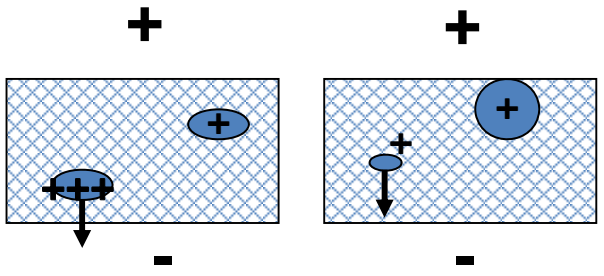
$\mu = v/E = \phi\ (q/m)$

No existe formulación matemática accesible que represente la movilidad electroforetica en función de parametros moleculares

Electroforesis un técnica empírica.



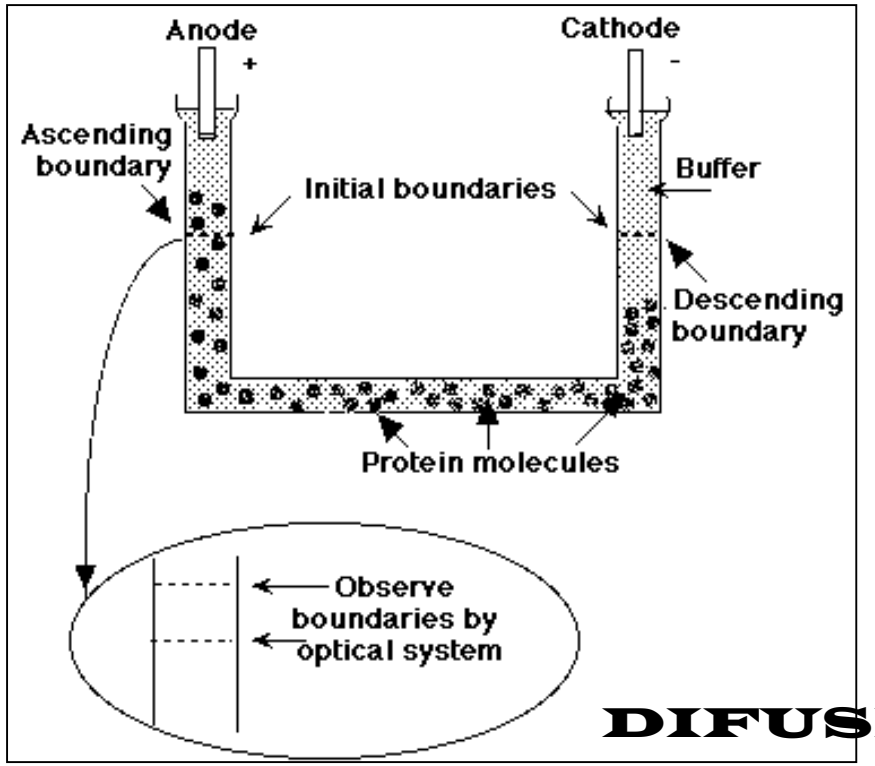




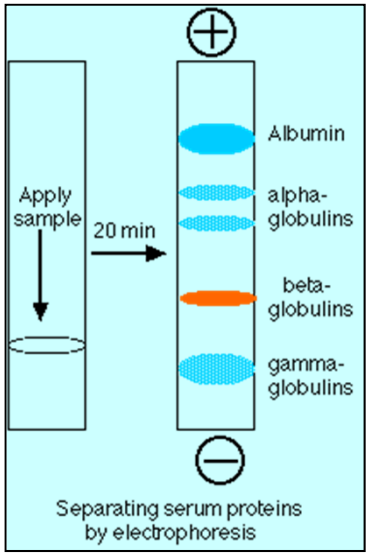
ELECTROFORESIS LIBRE: En solucion acuosa

TISELIUS
PRIMEROS
EXPERIMENTOS
ELECTROFORETICOS

PREMIO NOBEL 1948
“Estudios del plasma sanguíneo”

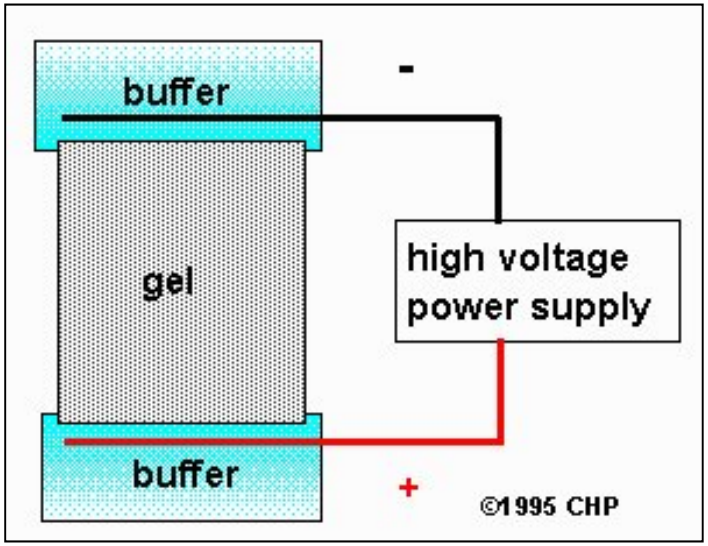


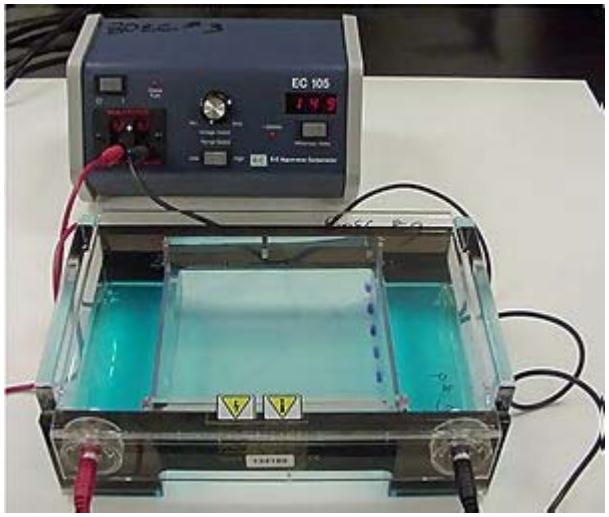
DIFUSION



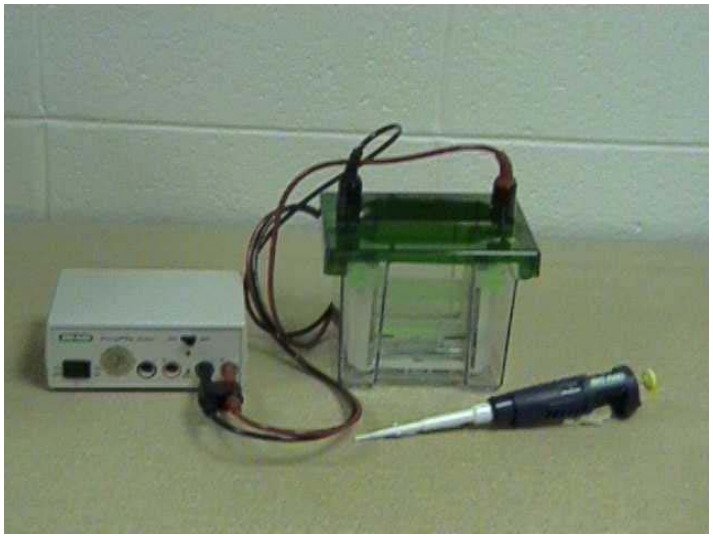
ELECTROFORESIS ZONAL➔En soporte
No restrictivo: papel, agarosa (poro>>molecula)
Restrictivo:poliacrilamida (mayor entramado)

EQUIPO BASICO:
FUENTE Y CUBETA
Un tampón determiado (pH)
$\mu=\phi$ (q/m) q=f (pH)





HORIZONTAL➔AGAROSA
ACIDOS NUCLEICOS



VERTICAL➔ACRILAMIDA (PAGE)
PROTEINAS

SEGÚN EL SOPORTE
PAPEL
CAPILAR
GELES.
AGAROSA
POLIACRILAMIDA (PAGE)

ELECTROFORESIS EN PAPEL
Moléculas pequeñas

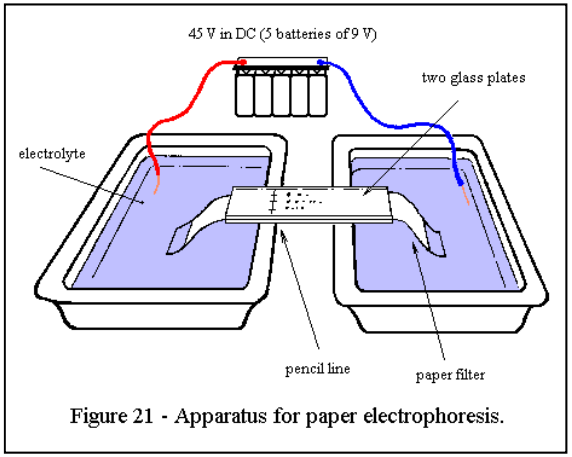
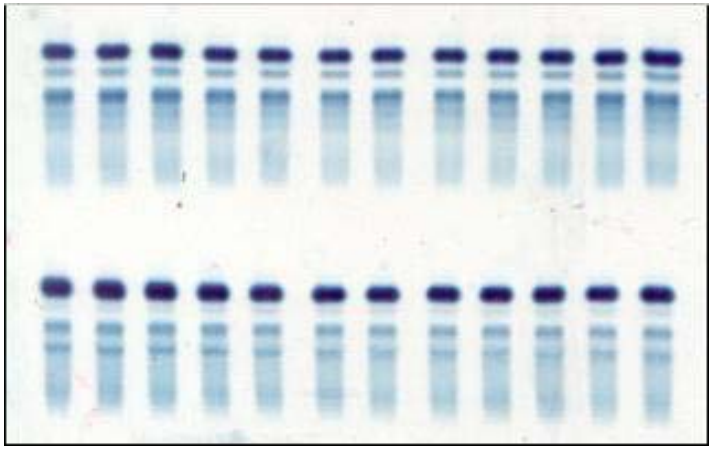


Figure 21 - Apparatus for paper electrophoresis.

ACETATO DE CELULOSA



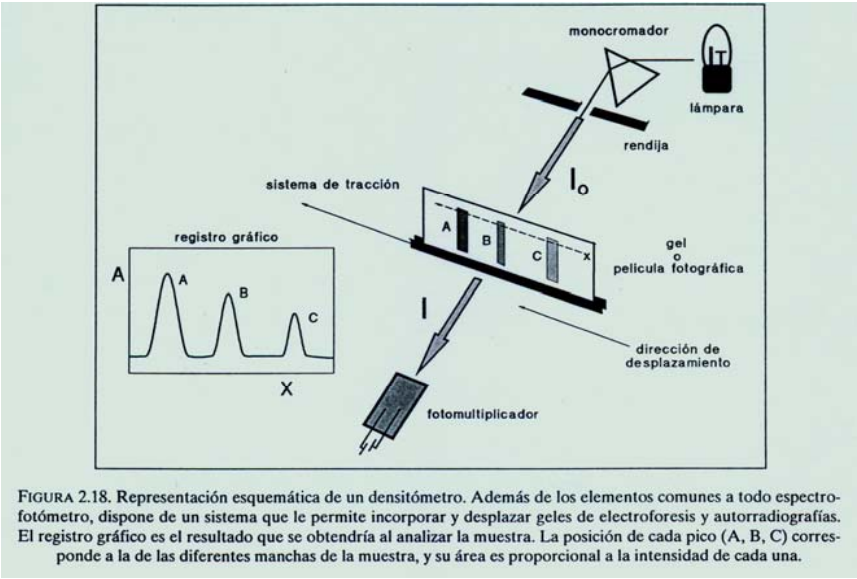
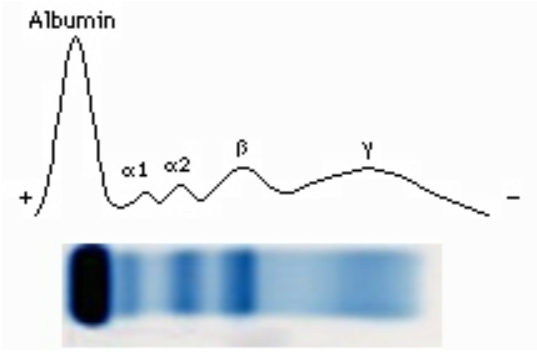
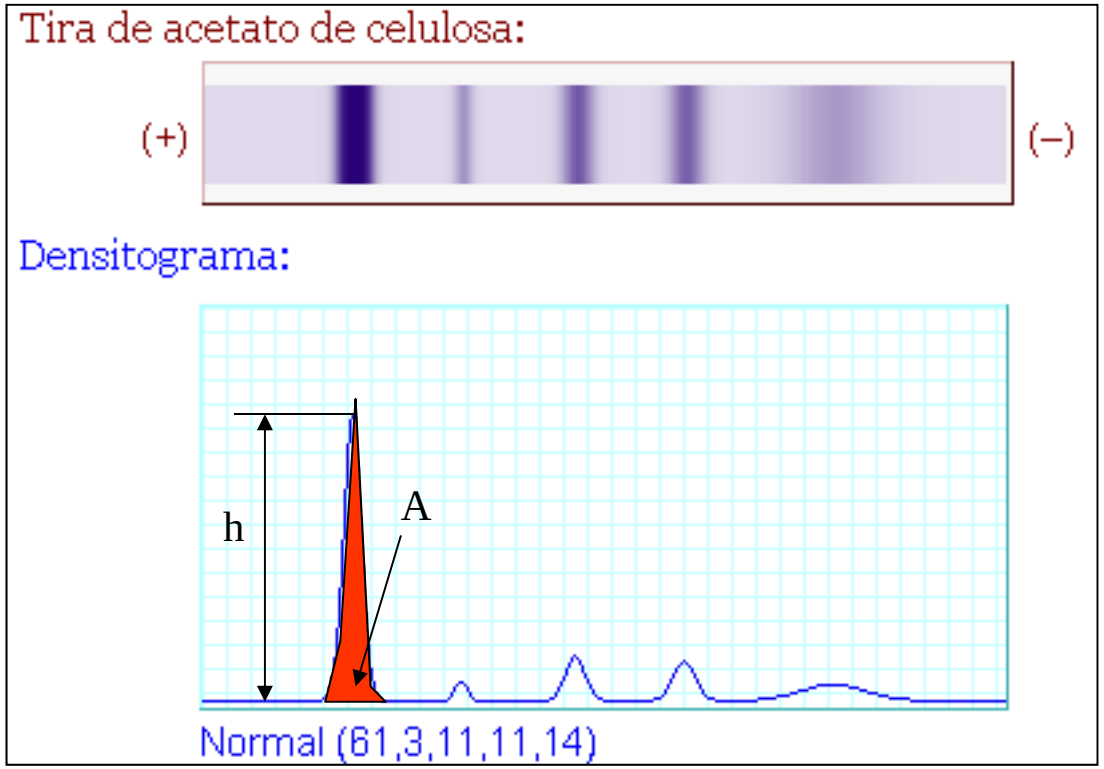


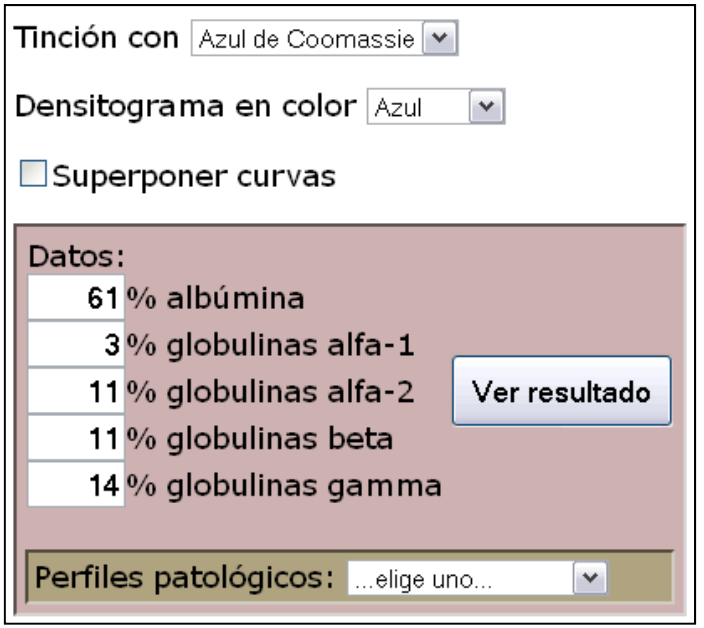
FIGURA 2.18. Representación esquemática de un densitómetro. Además de los elementos comunes a todo espectrofotómetro, dispone de un sistema que le permite incorporar y desplazar geles de electroforesis y autorradiografías. El registro gráfico es el resultado que se obtendría al analizar la muestra. La posición de cada pico (A, B, C) corresponde a la de las diferentes manchas de la muestra, y su área es proporcional a la intensidad de cada una.

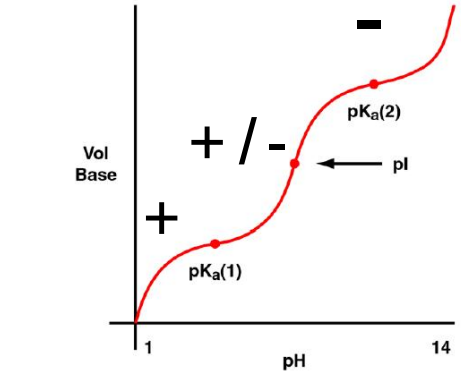
densitometría

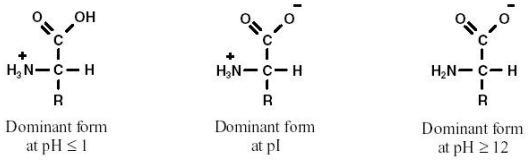


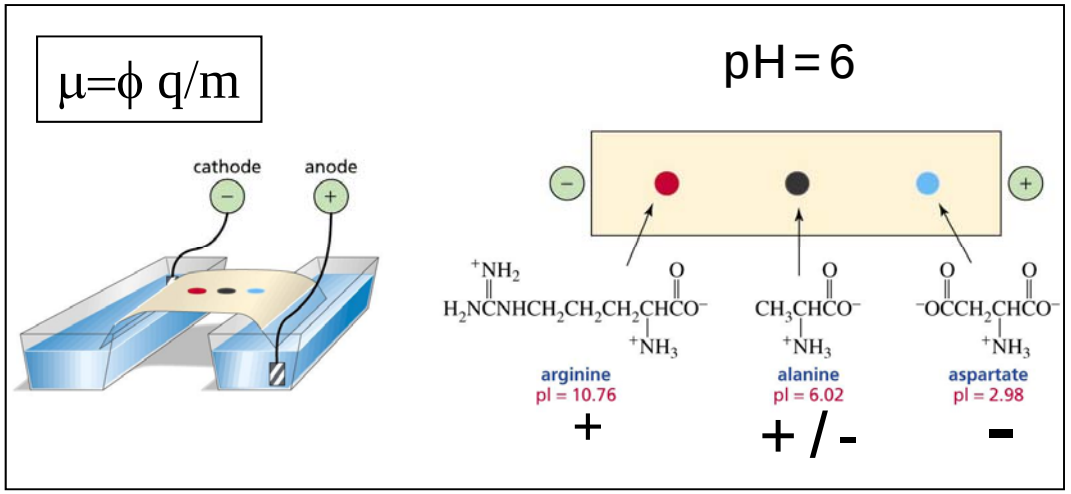
DENSITOGRAMA➔ METODO DE CUANTIFICACION ELECTROFORETICA

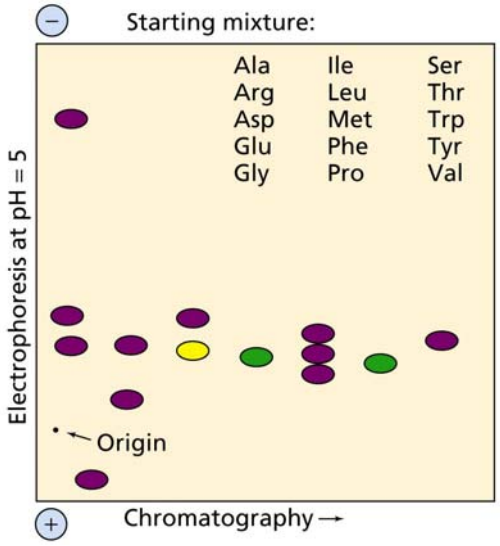


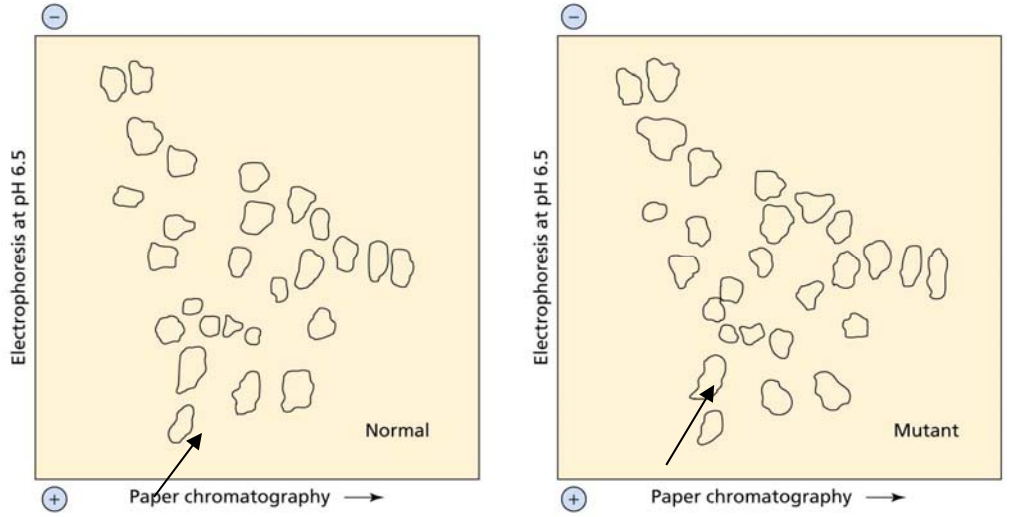




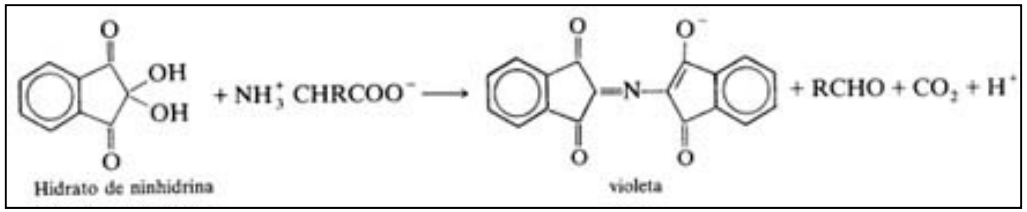


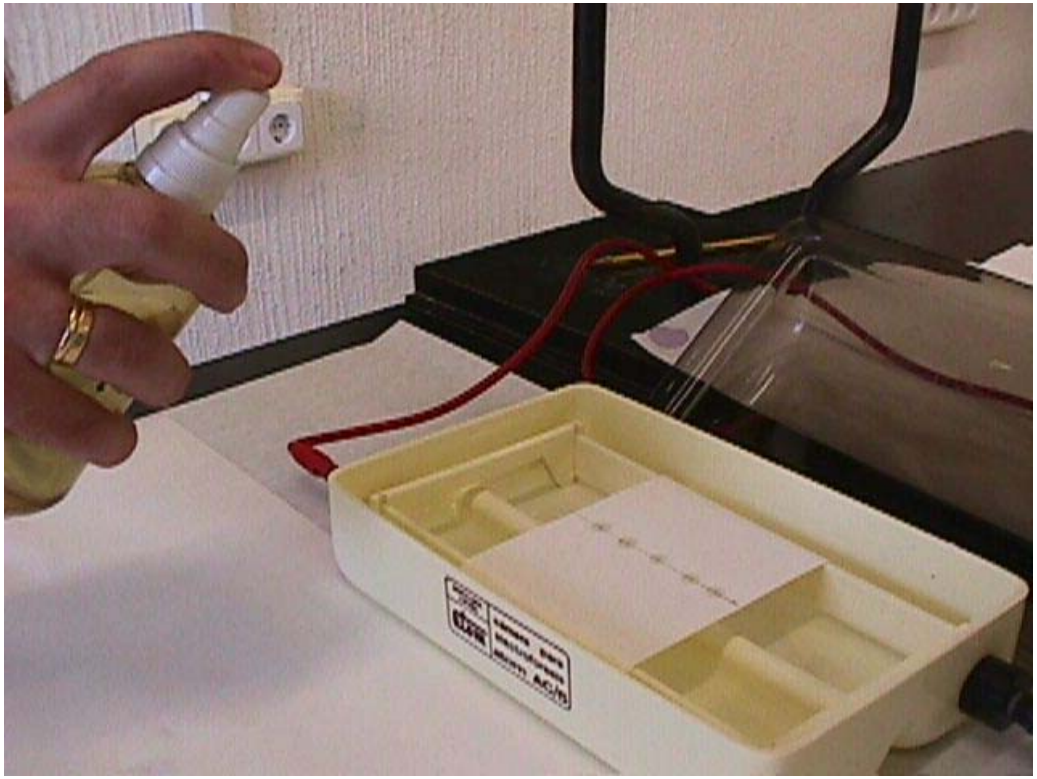


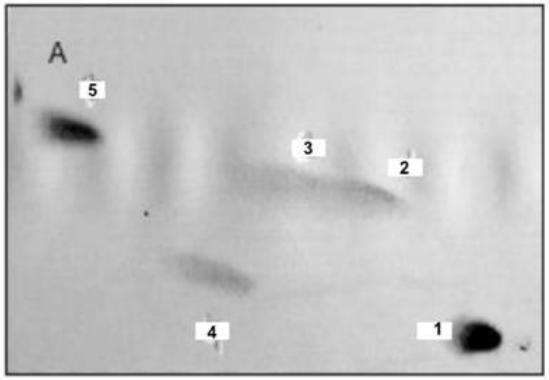


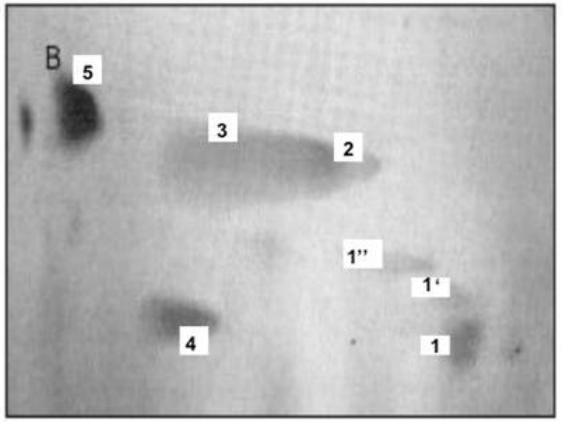


SEPARACION BIDIMENSIONAL: CROMATOGRAFIA+ELECTROFORESIS


OH
OH
+ NH$_3^+$ CHRCOO$^-$
+ RCHO + CO$_2$ + H$^+$
Hidrato de ninhidrina
violeta

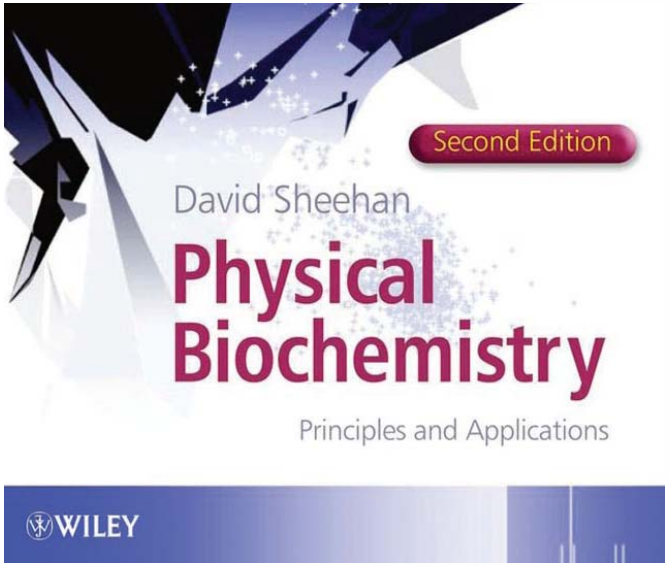


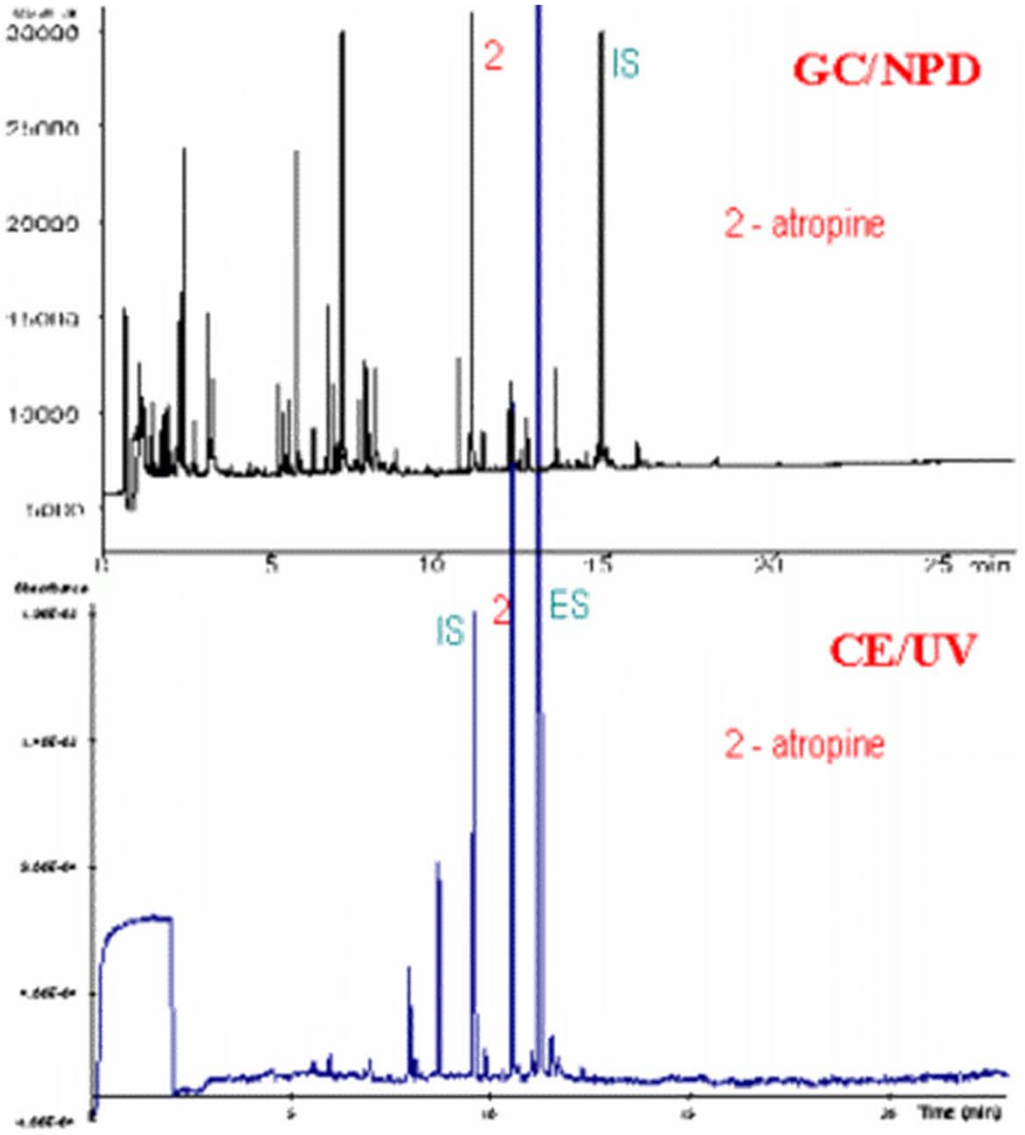

A
5
3
2
4
1


B
5
3
2
1''
1'
4
1

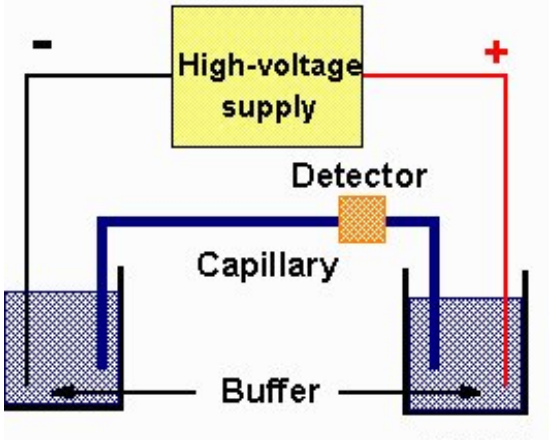
ALTO VOLTAJE!
(5-50KV)

ELECTROFORESIS CAPILAR



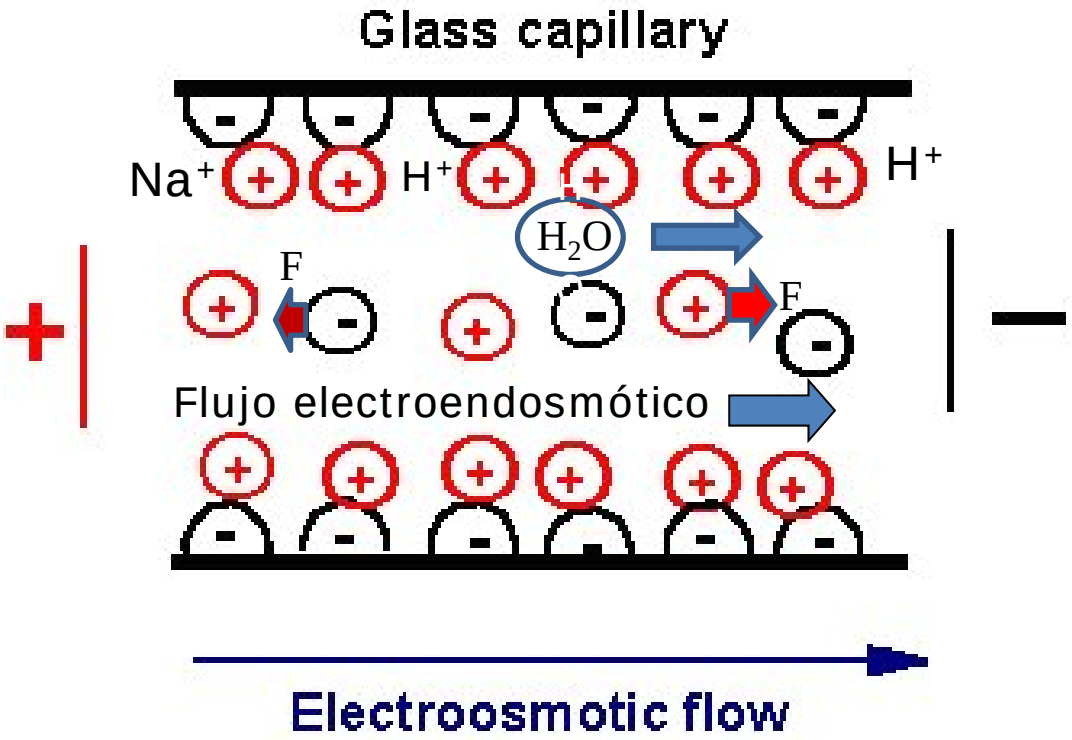


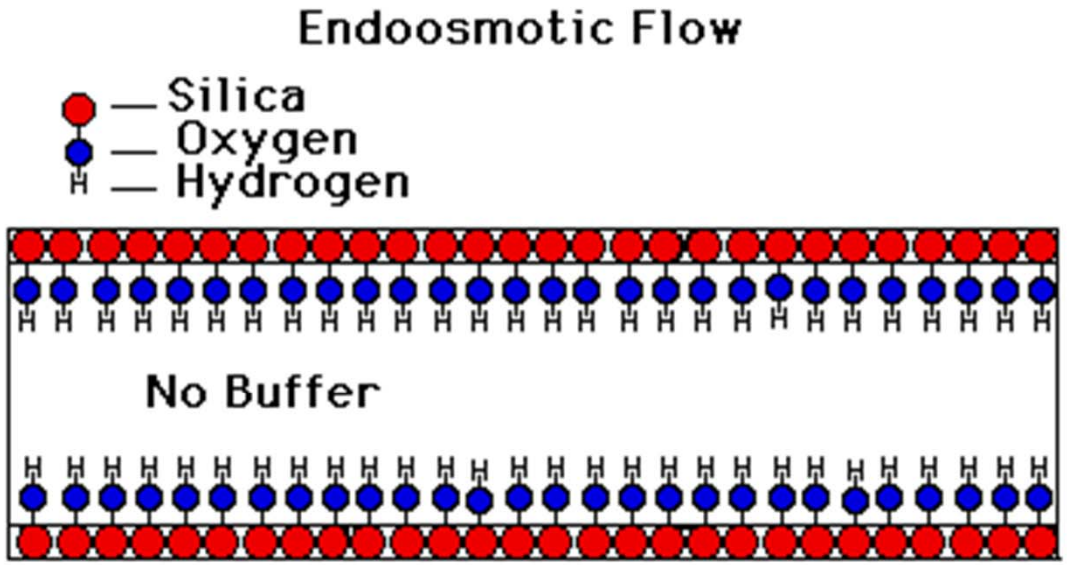
Free solution CE
Capilar Gel Electroforesis

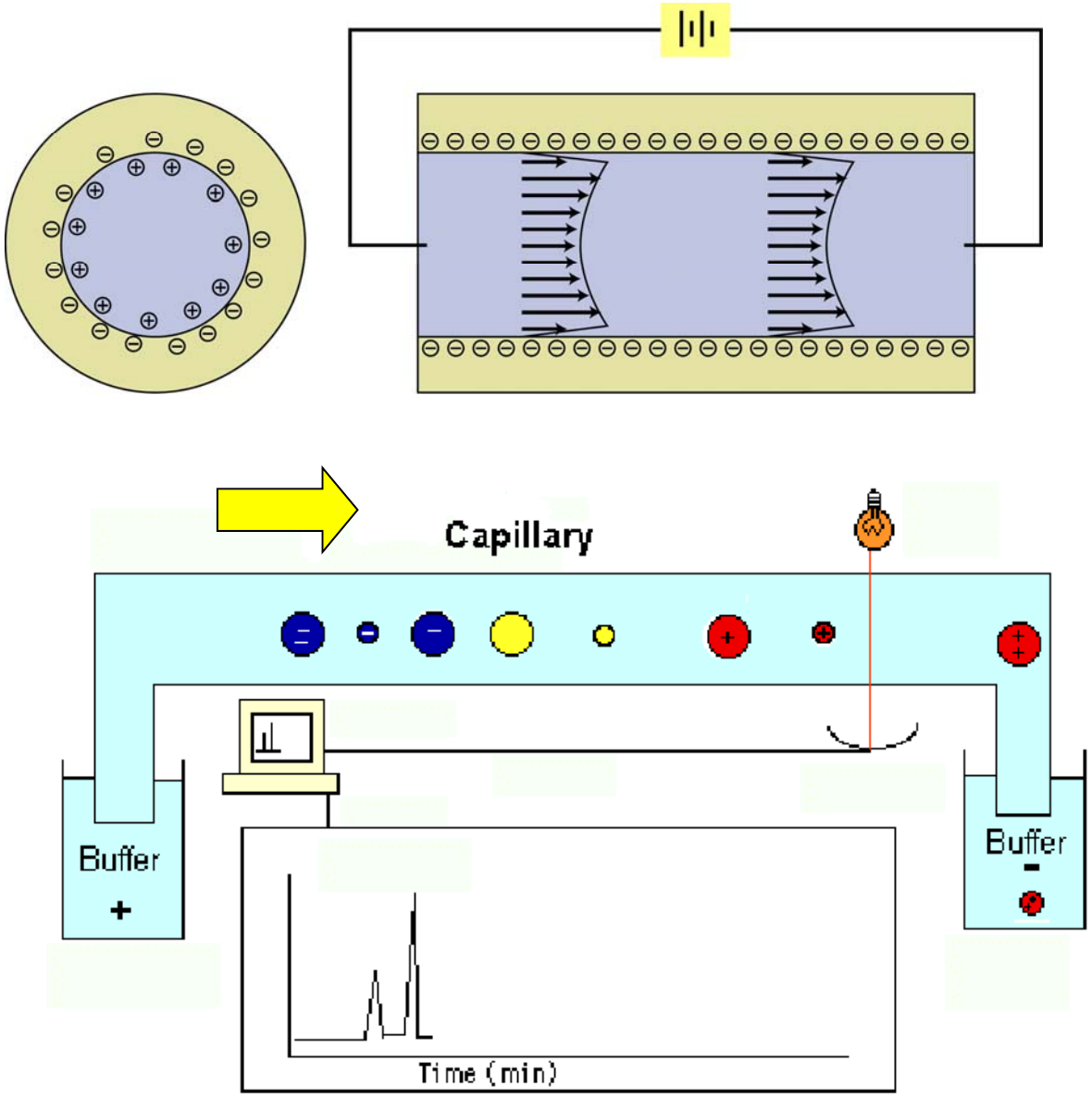


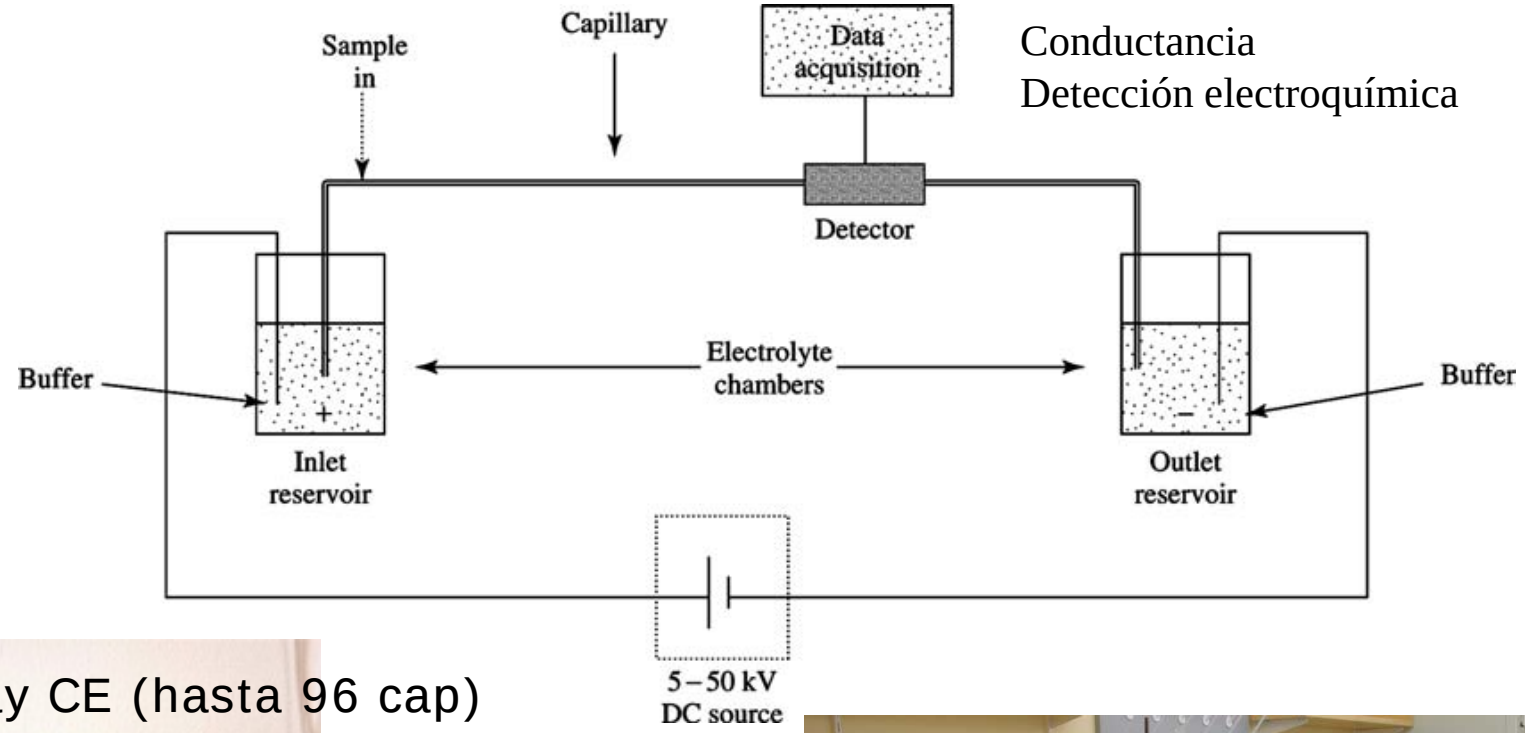
Ø=20-200 μm
V= hasta 50.000V

Pared de capilar compuesto de silicatos
Grupos silanol : $R_3Si\text{-}OH \leftrightarrow R_3Si\text{-}O^- + H^+$



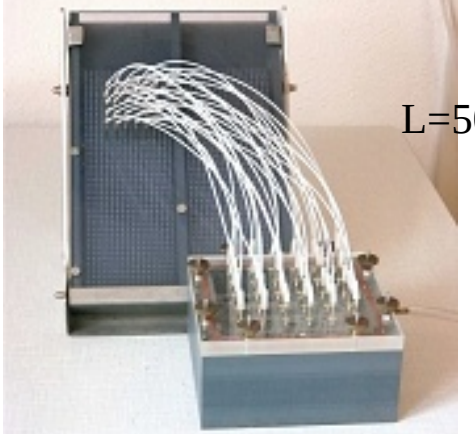

Endoosmotic Flow
Silica
Oxygen
Hydrogen
No Buffer


Capillary
Buffer
+
Buffer
-
Time (min)



Conductancia
Detección electroquímica

Array CE (hasta 96 cap)



L=50-100cm

ϕi=10-100μm

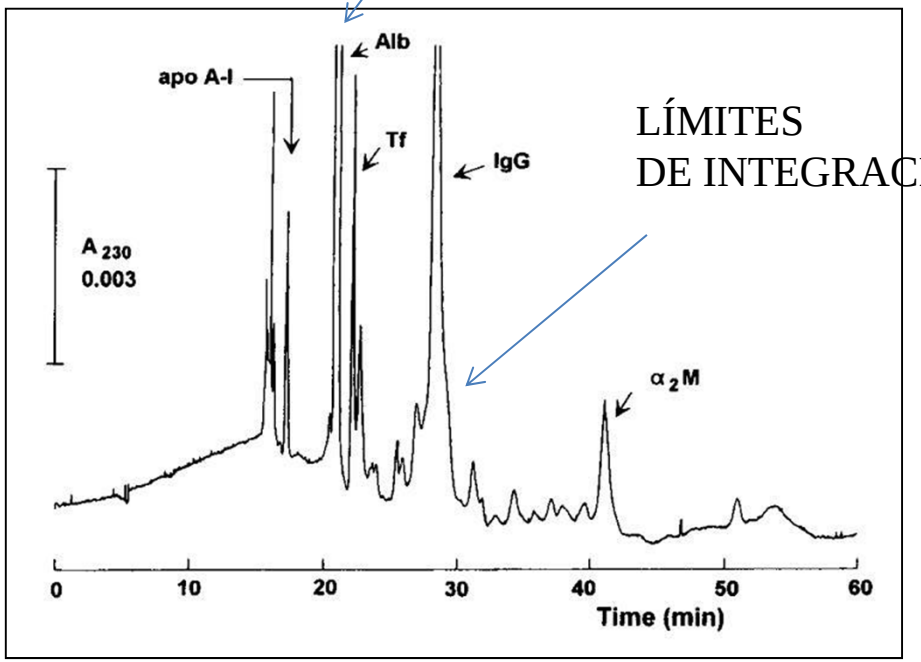
Rápido y cuantificable
¡HAY QUE OPTIMIZAR CONDICIONES DE SEPARACIÓN!

Optimizing CE Separations-POINT method

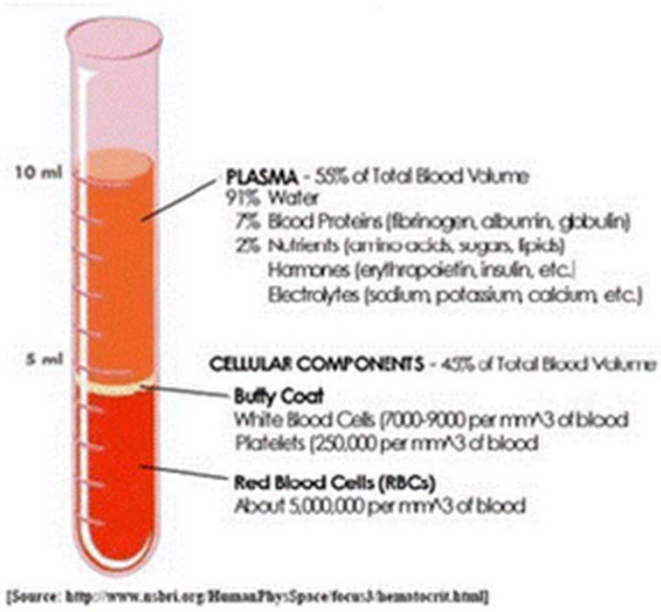
- pH
  - First paramater to control
  - Effects EOF and mobility (charge)
- Organic Solvent
  - Analyte solvation
- Interacting agent
  - Ion-pairing, solvation, etc.
- Non-aqueous Conditions
  - Solvation and charge
- Temperature
  - Solvation, chemical equilibria

Proteinas del plasma
(incluye los componentes de la coagulación)

SATURACIÓN DE ALBUMINA



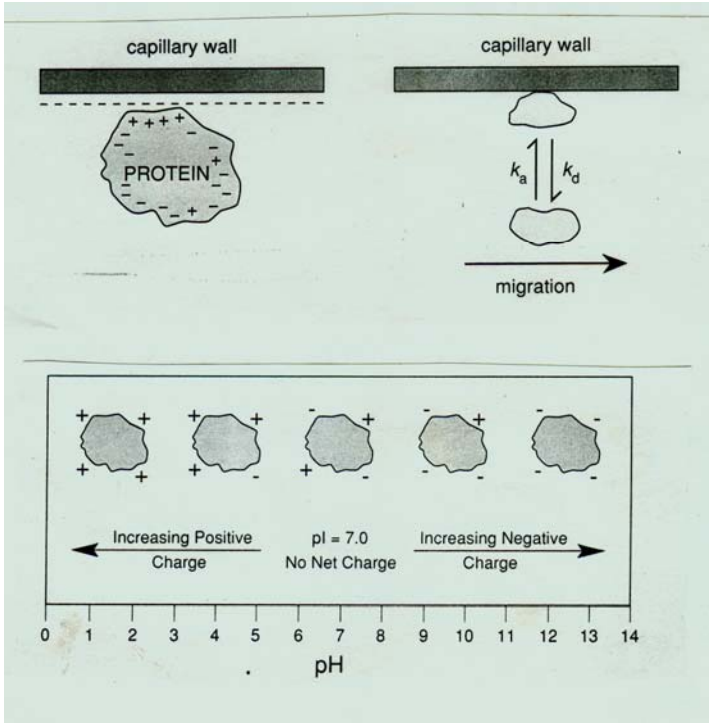
LÍMITES
DE INTEGRACIÓN



Grupos silanol :

$R_3Si\text{-}OH \leftrightarrow R_3Si\text{-}O^- + H^+$

A menor pH, más protonada la pared, menos carga y menor EOF.
En teoria, mejor resolucion...pero a pH bajo proteinas con más carga + interaccionan más con la pared…
Hay que optimizar condiciones!!



Aquí: A mayor pH mejoramos resolución!

Incrementando fuerza iónica disminuye interacción con pared y mejora resolución..

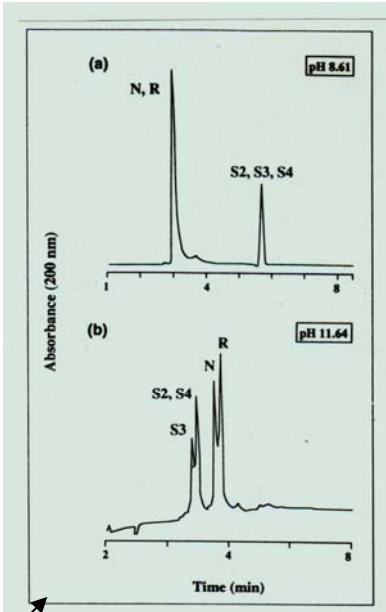


Figure 3
The pH-dependent separation of peptides with 'shuffled' amino acid sequences. The model peptide system used was a 12 amino acid sequence, KTNYC-TKPQKSY, from residues 101–112 of the thyroid-stimulating-hormone receptor. Separation was carried out in a 50 µm × 20 cm capillary in 100 mM borate, 10 mM diaminopentane, with pH adjusted to (a) 8.61 and (b) 11.64 with NaOH. N, R and S represent the 'native', 'reverse' and 'shuffled' peptide sequences, respectively. The instrumentation used was a Beckman P/ACE 2050 with detection at λ = 200 nm.

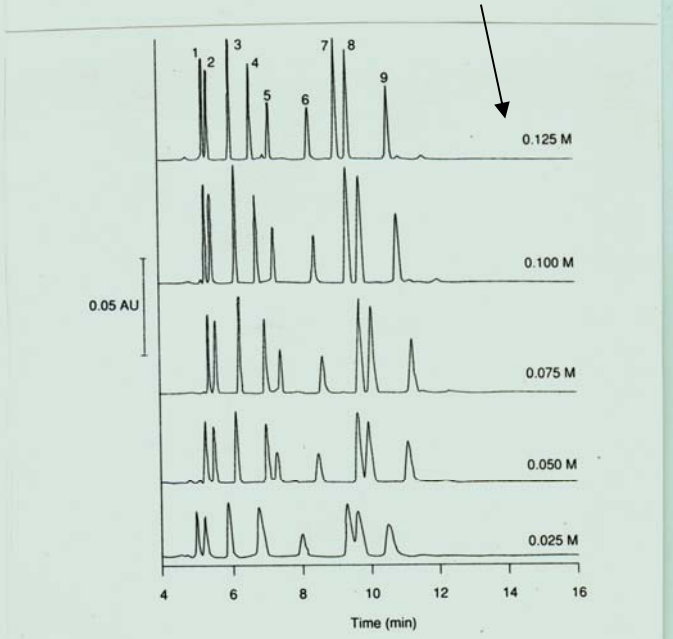
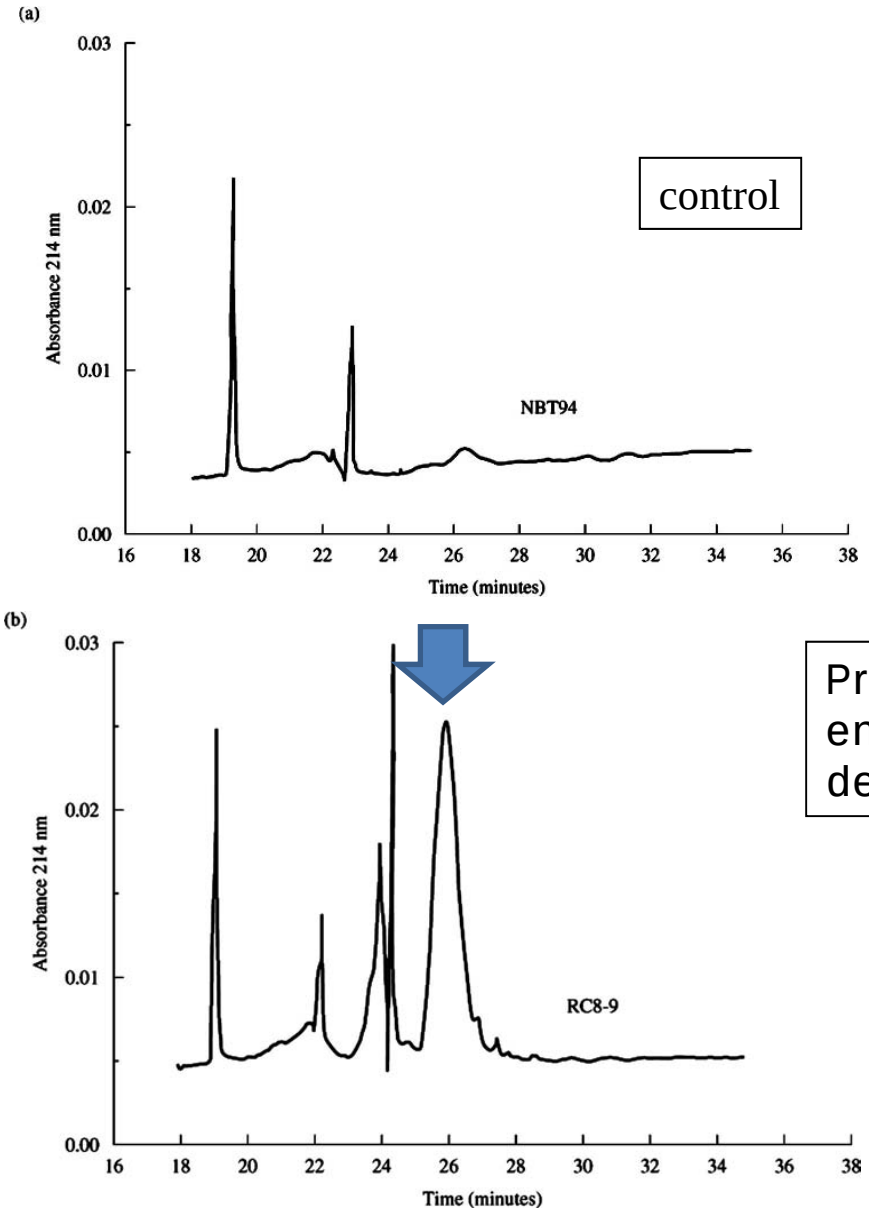


***Figure 1-26**. Effect of the buffer ionic strength on peak shape and migration time of peptides. Buffer: sodium phosphate, pH 2.44; 30 kV; 20°C; 200 nm; 57 (50) cm × 75 µm capillary. Peak identification: (1) dynorphin; (2) bradykinin; (3) angiotensin II; (4) TRH; (5) LHRH; (6) bombesin; (7) leu-enkephalin; (8) met-enkephalin; (9) oxytocin. From McLaughlin* et al., Beckman Technical Information Bulletin TIBC-106 *(1991).*



control

Priones identificados en extracto de cerebro de oveja infectada

"Plasma creatinine and creatine quantification by capillary electrophoresis diode array detector"
Analytical Biochemistry vol342 (2005) 186–193

Sangre + anticoagulante

⊕

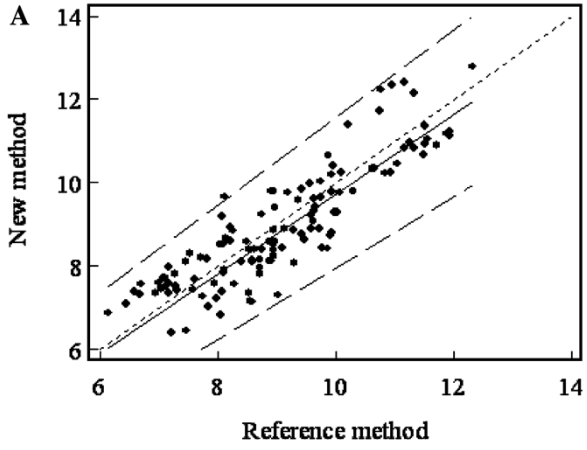
3000g x 5min

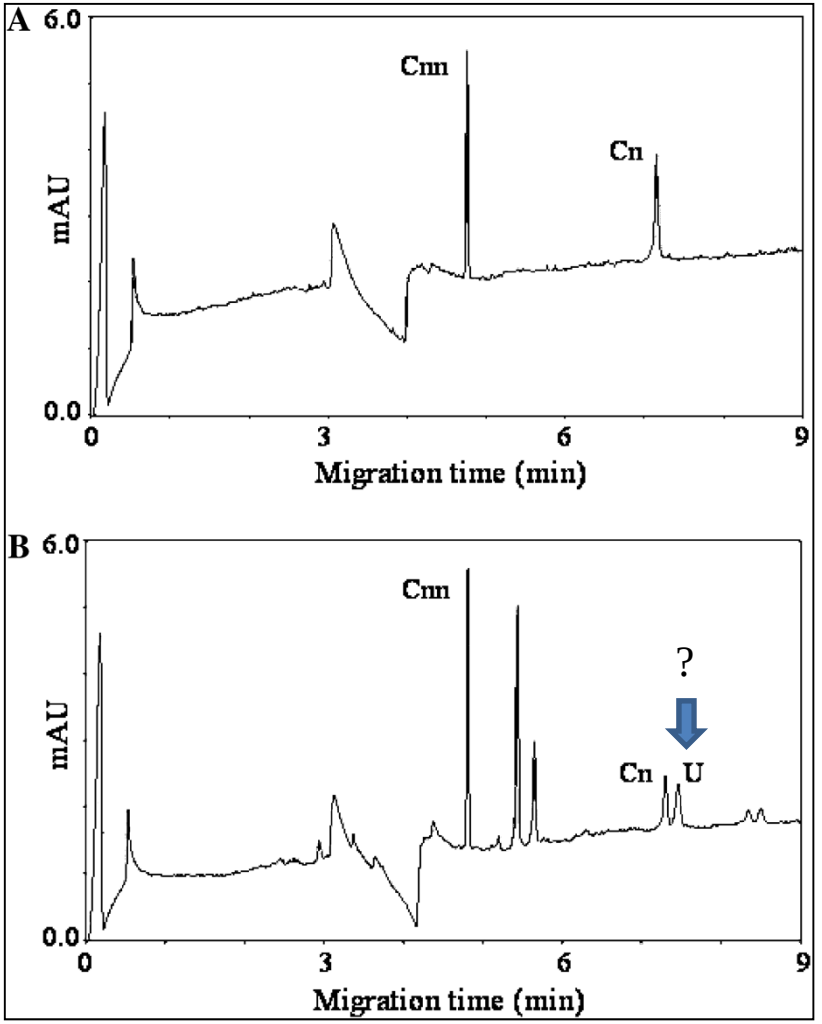
PLASMA

TCA 5%

⊕

SOBRENADADANTE





75mM Tris/fosfato pH=2,25

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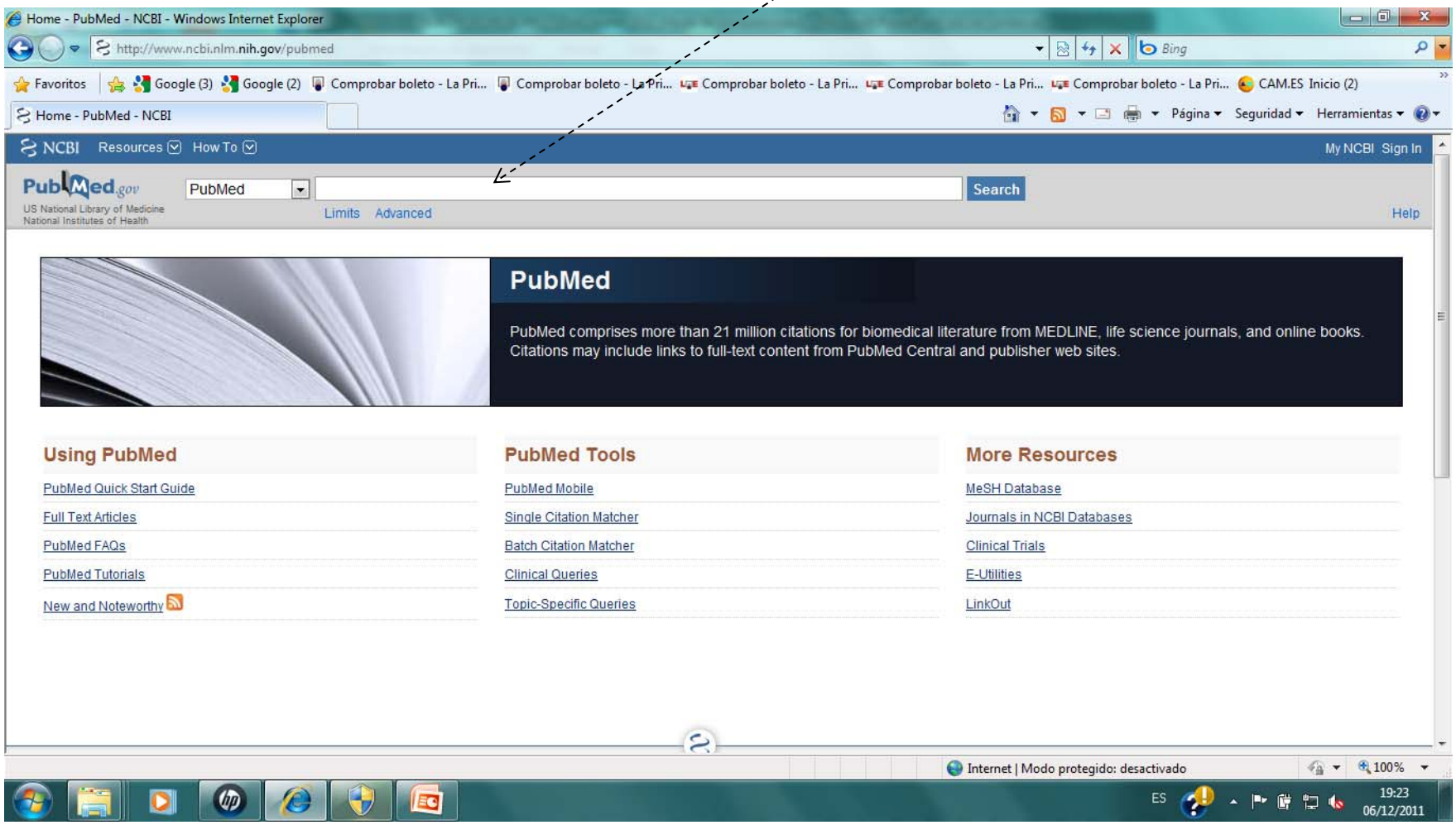
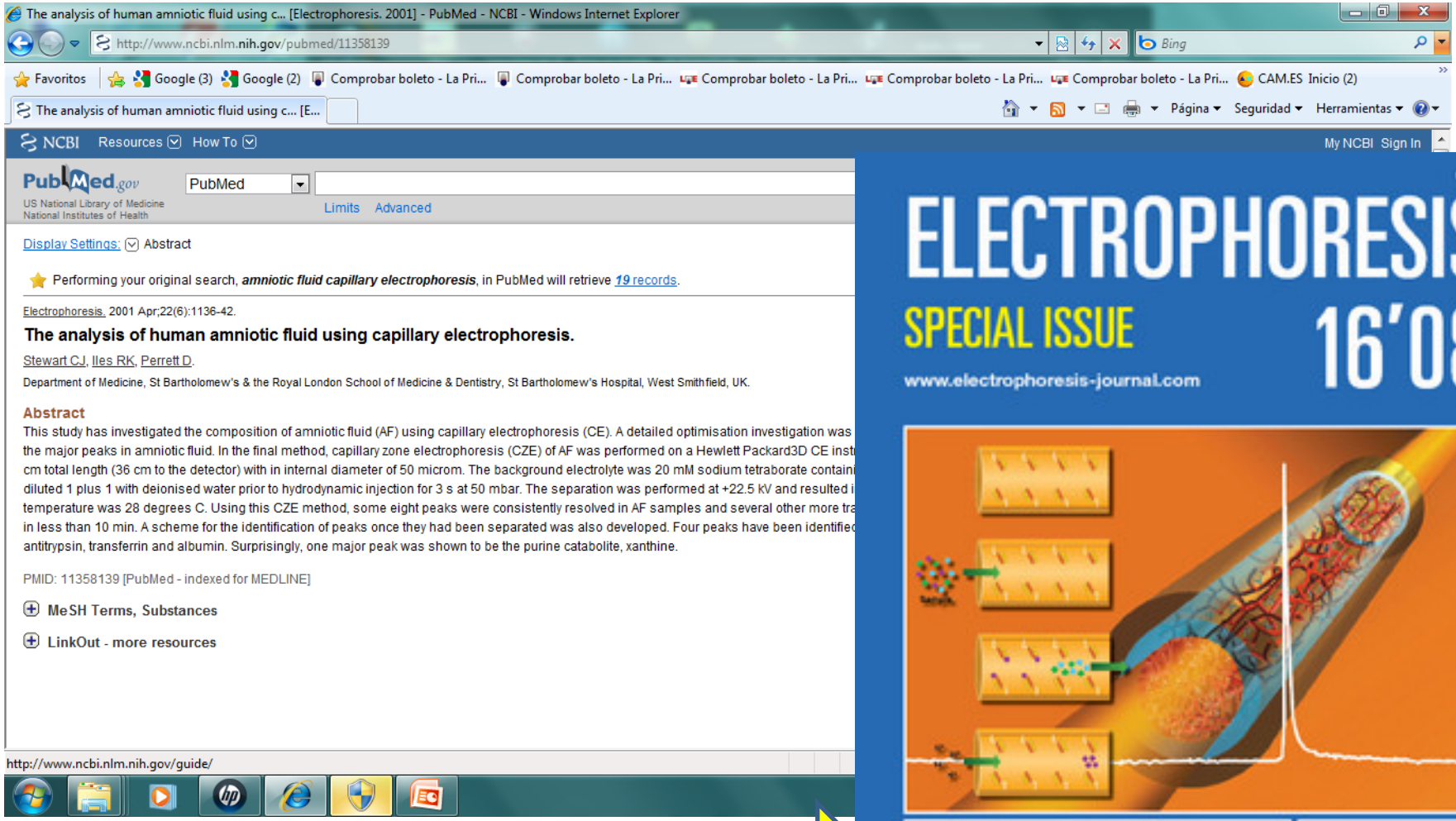
Capillary electrophoresis





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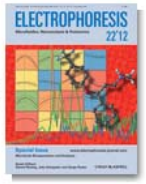
ELECTROPHORESIS

Short communication

Capillary gel electrophoresis for precise protein quantitation

Claudia Cianciulli, Thomas Hahne, Hermann Wätzig*

Article first published online: 12 SEP 2012
DOI: 10.1002/elps.201200177



ELECTROPHORESIS
Special Issue: MicroScale Bioseparations and Analyses
Volume 33, Issue 22, pages 3276–3280, November 2012

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Keywords:
CGE; Monoclonal antibody; Protein; Quantitation; SDS-CGE

CGE, also known as SDS-CGE, is being established in the pharmaceutical industry replacing SDS-PAGE. In most cases, the method is applied for the identity and purity control of proteins, for example monoclonal antibodies. In order to quantify these components with sufficient precision using the same quality control method, a RSD for the quantitative analysis under 2% is required. A reliable and highly precise CGE method could be obtained after thorough optimization. It was crucial to increase the sample concentration and the injection volume in order to achieve sufficiently high S/N ratios (>70). The application of hydrodynamic injection is beneficial for the precision of the method compared to the traditionally used electrokinetic one. Linearity was demonstrated and LOD and LOQ were estimated. Both injection modes were compared in long series runs (n = 48). Furthermore, the use of an internal standard was investigated. Thus, the RSD% of the migration time was reduced from 0.9 to 0.2% and the RSD% of peak areas was greatly improved. However, the normalization to the total area further reduced the influence of the injection error. RSD% for the peak area ratios of typically between 1 and 2% was provided.

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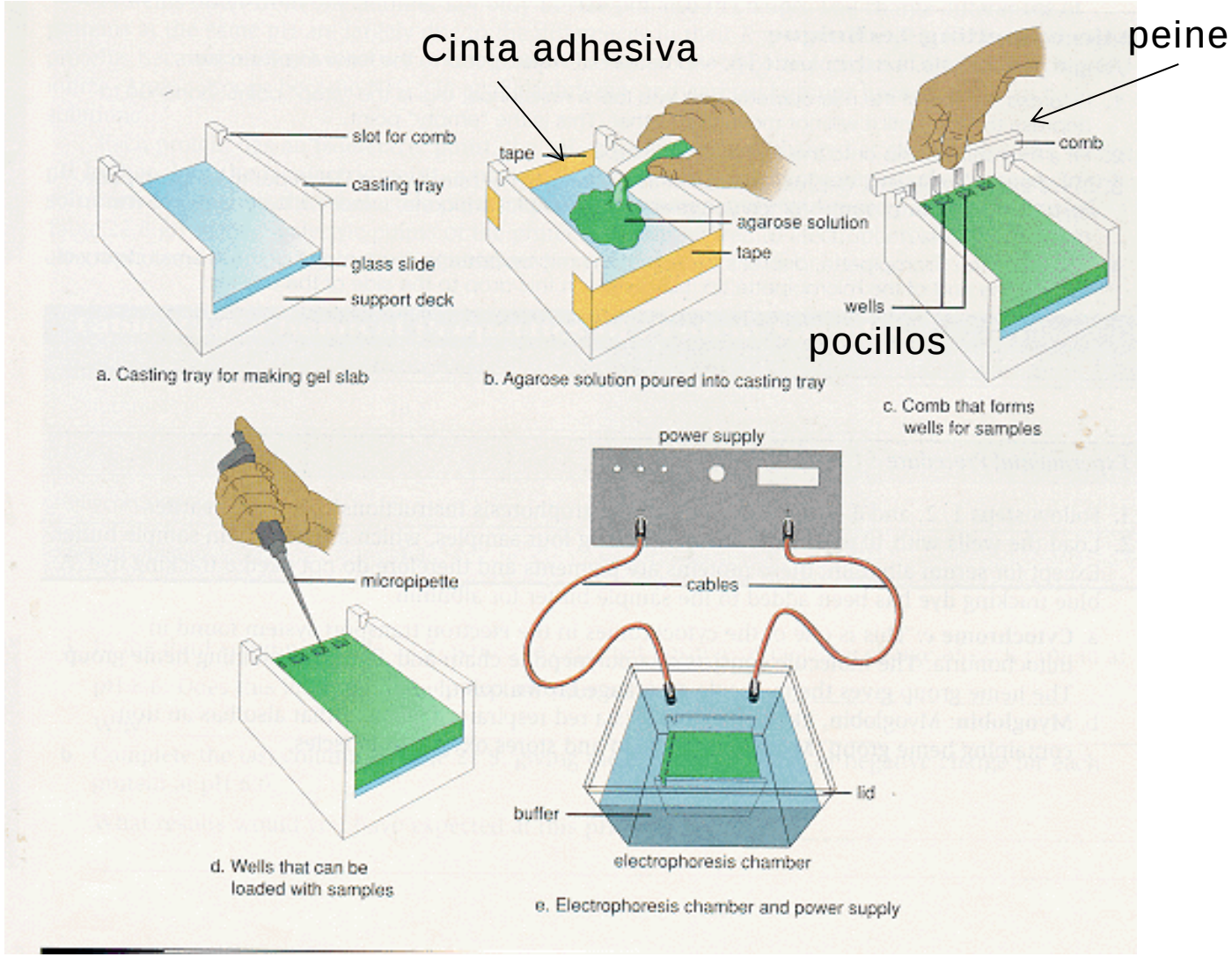
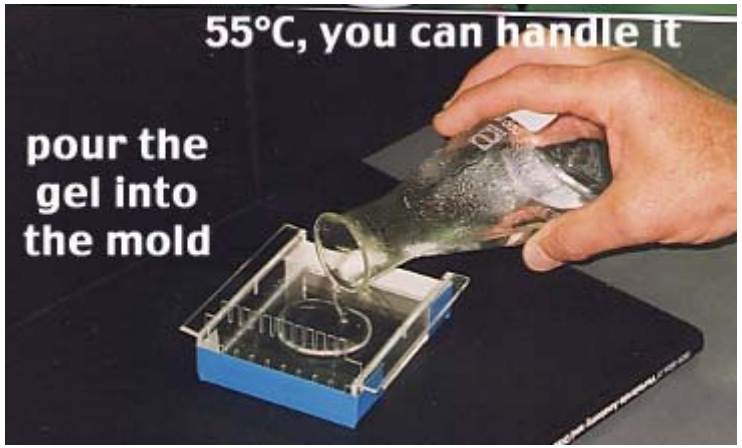
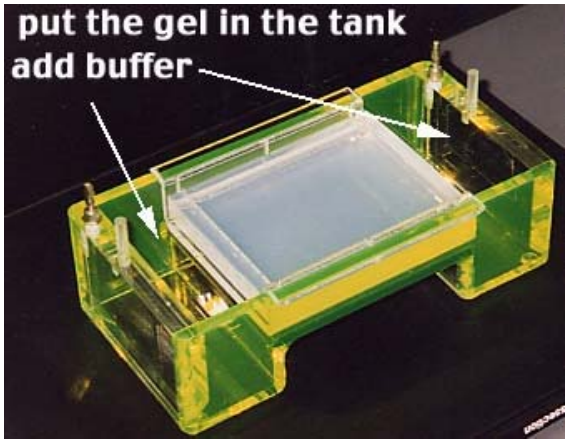
ELECTROFORESIS EN GELES , MATRICES HIDRATADAS ESTABLES

AGAR, **AGAROSA**: ACIDOS NUCLEICOS

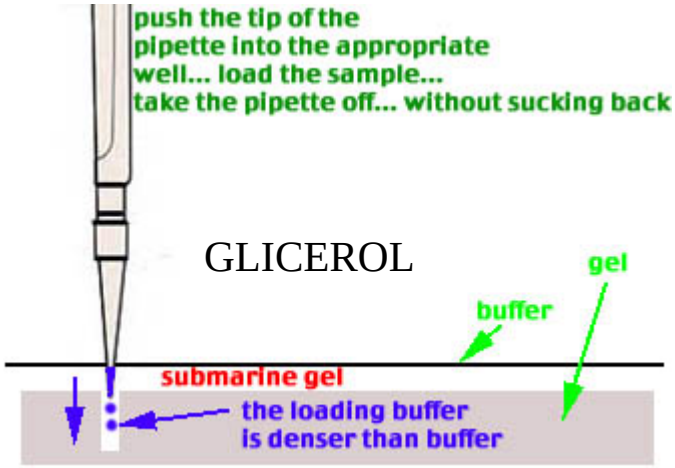
POLIACRILAMIDA (PAGE): PROTEINAS





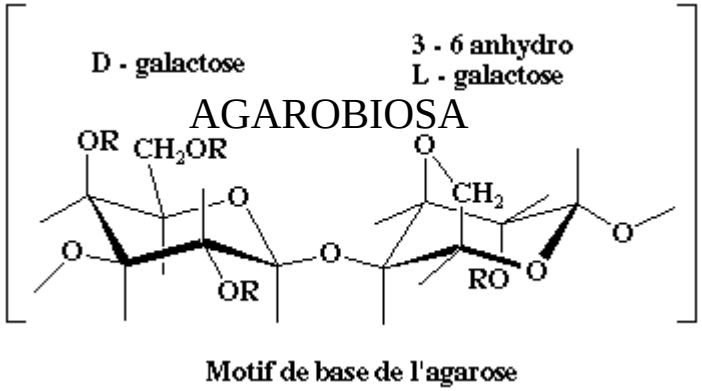


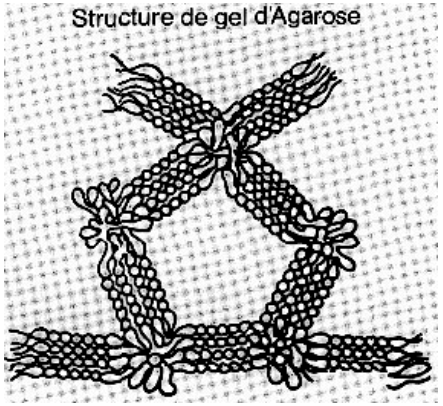
PREFORMAR EL GEL

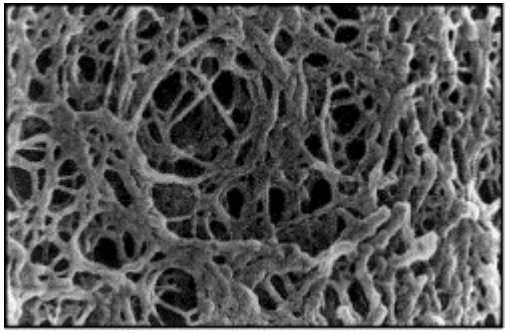


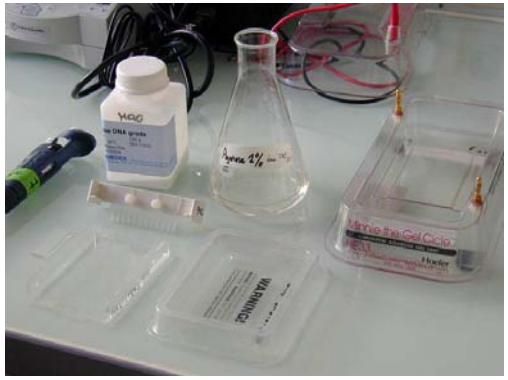
APLICAR LA MUESTRA

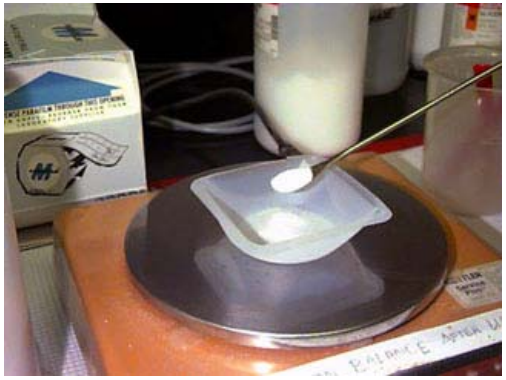
GELES DE AGAROSA: ACIDOS NUCLEICOS

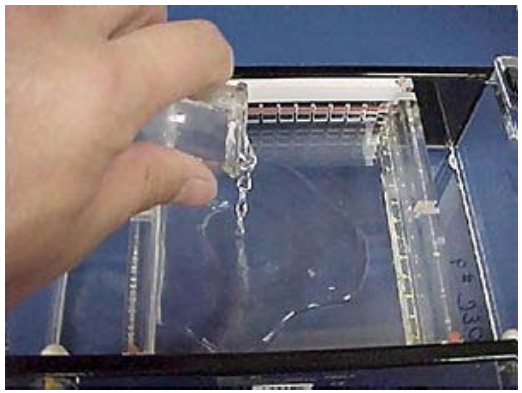


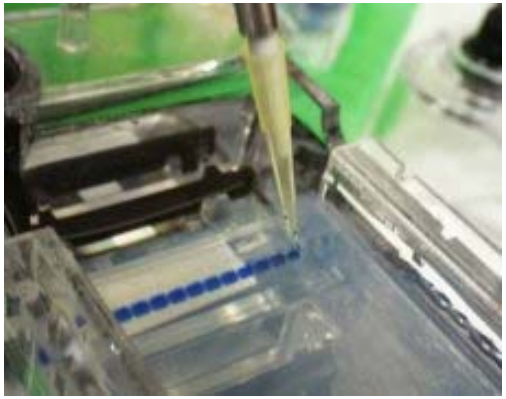


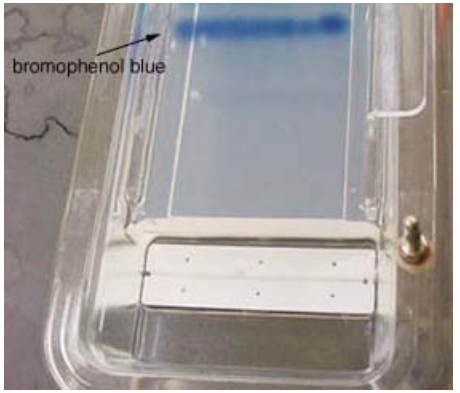


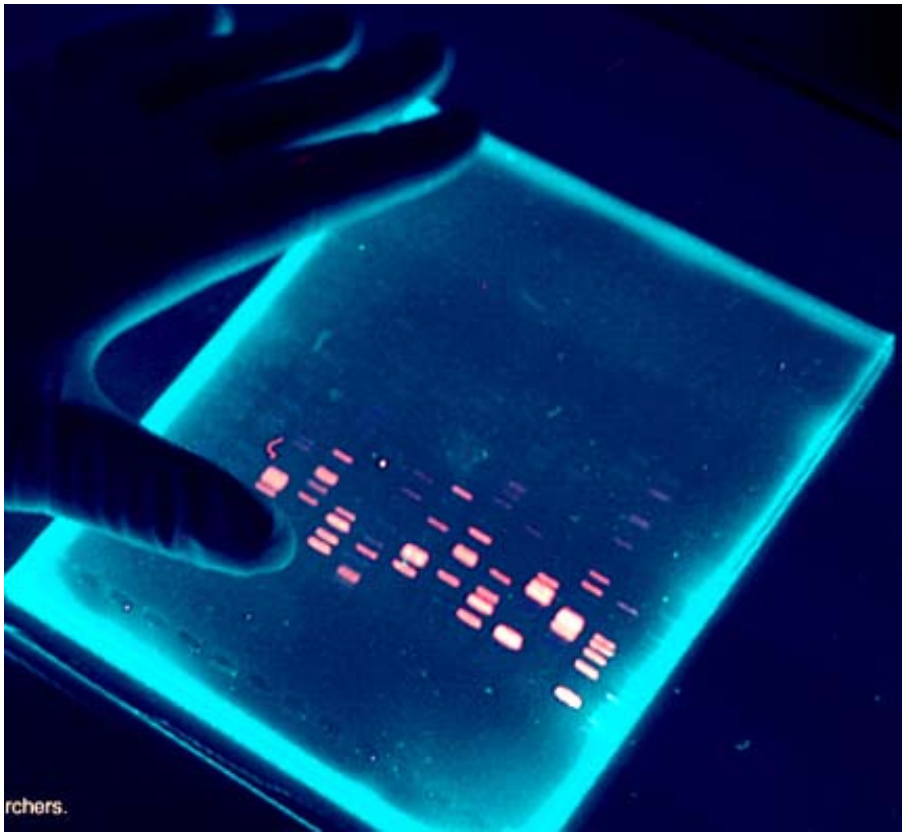


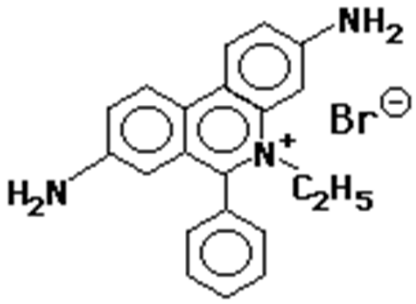




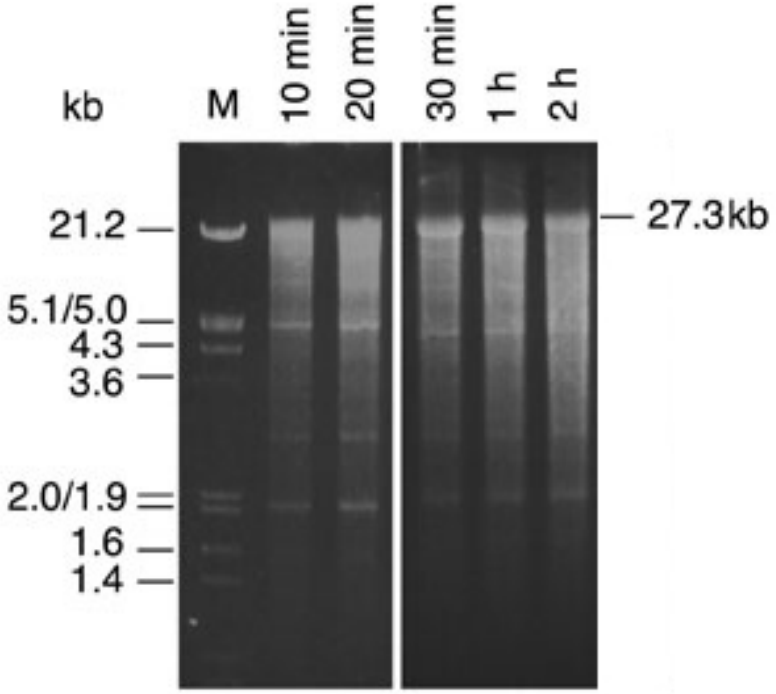




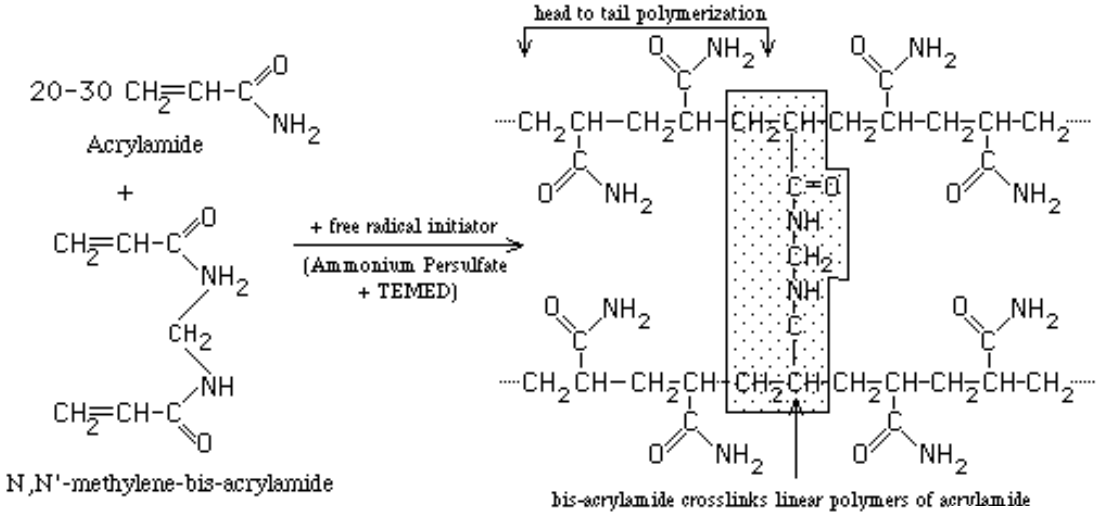




Ethidium Bromide

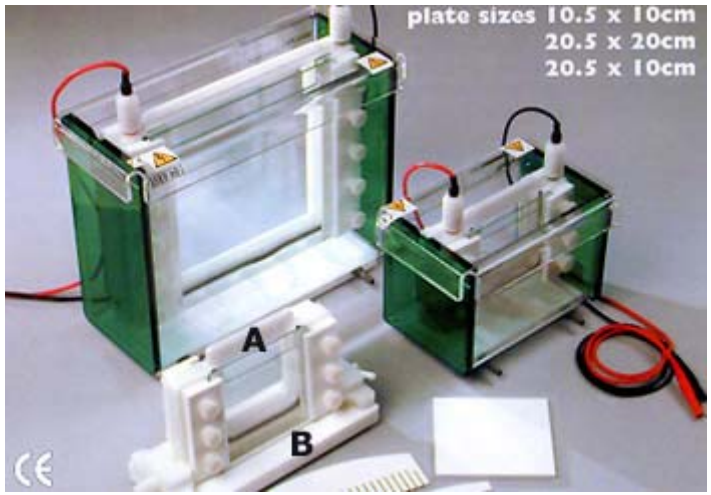


ELECTROFERESIS EN GELES DE POLIACRILAMIDA: PAGE



$S_2O_8(NH_4)_2$ ⟹ $S_2O_8^{2-}$ ➔ $2\ SO_4^-$

Persulfato de amonio radical sulfato

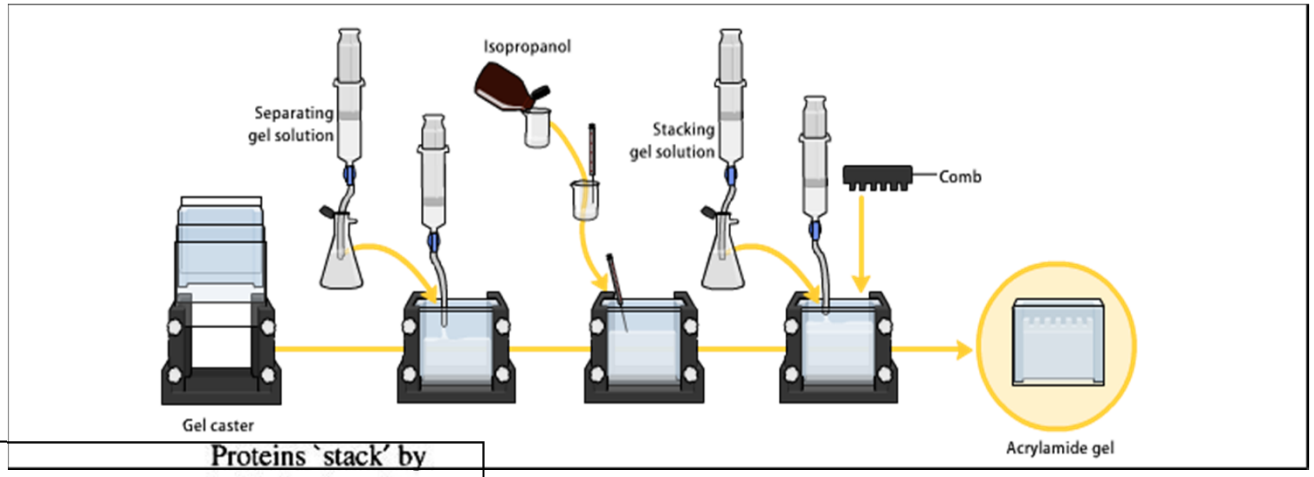


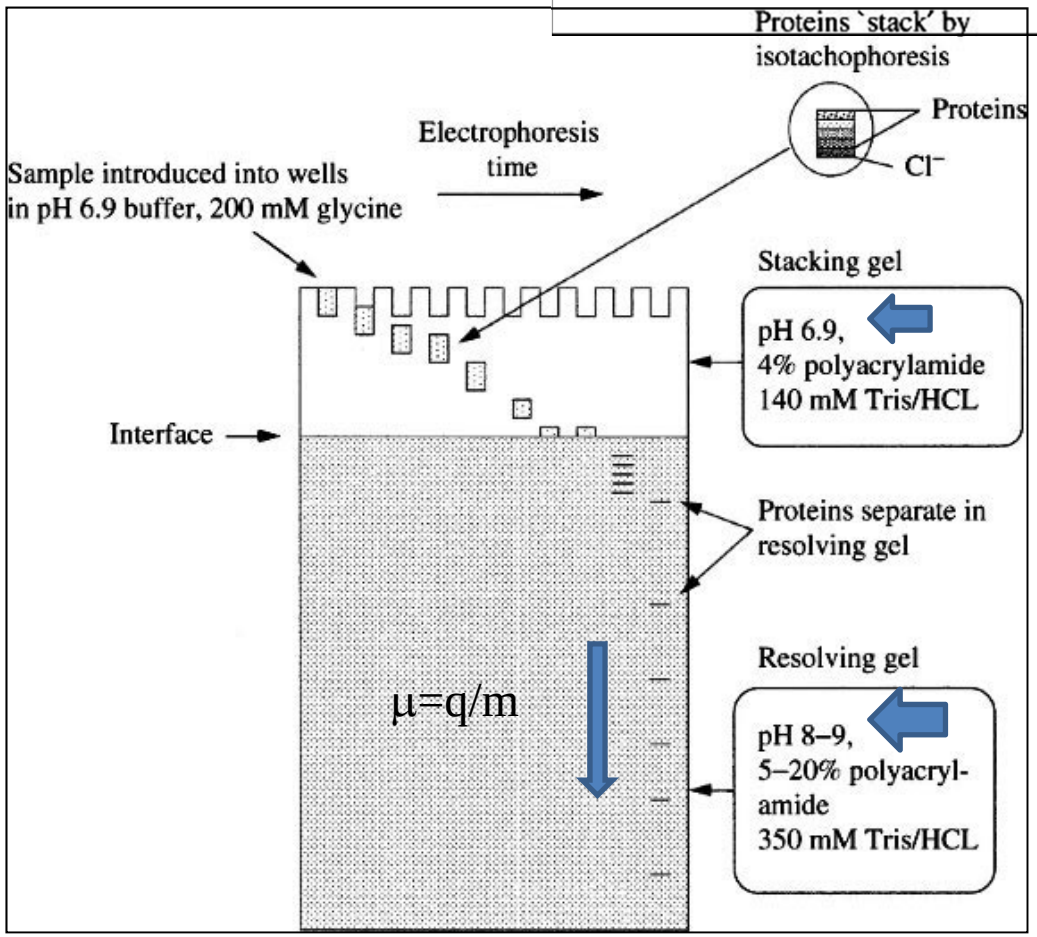
CATALIZADORES DE LA POLIMERIZACION

TEMED= NNN'N' TETRAMETILETILENEDIAMINA

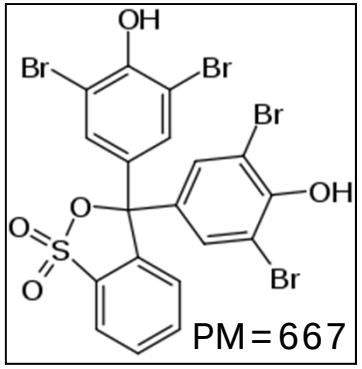
PERSULFATO DE AMONIO

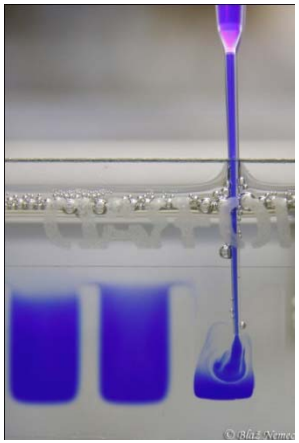
GELES DISCONTÍNUOS:
STACKING GEL(pH6)
RESOLVING GEL(pH8)

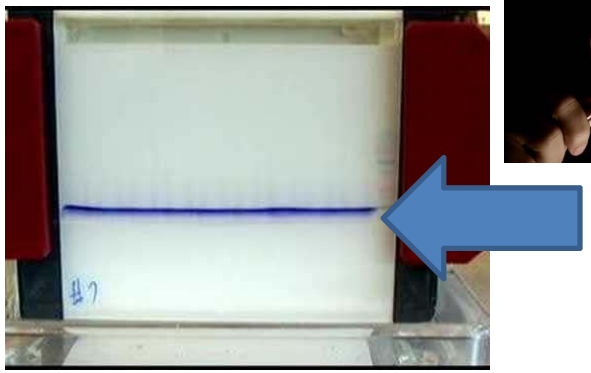




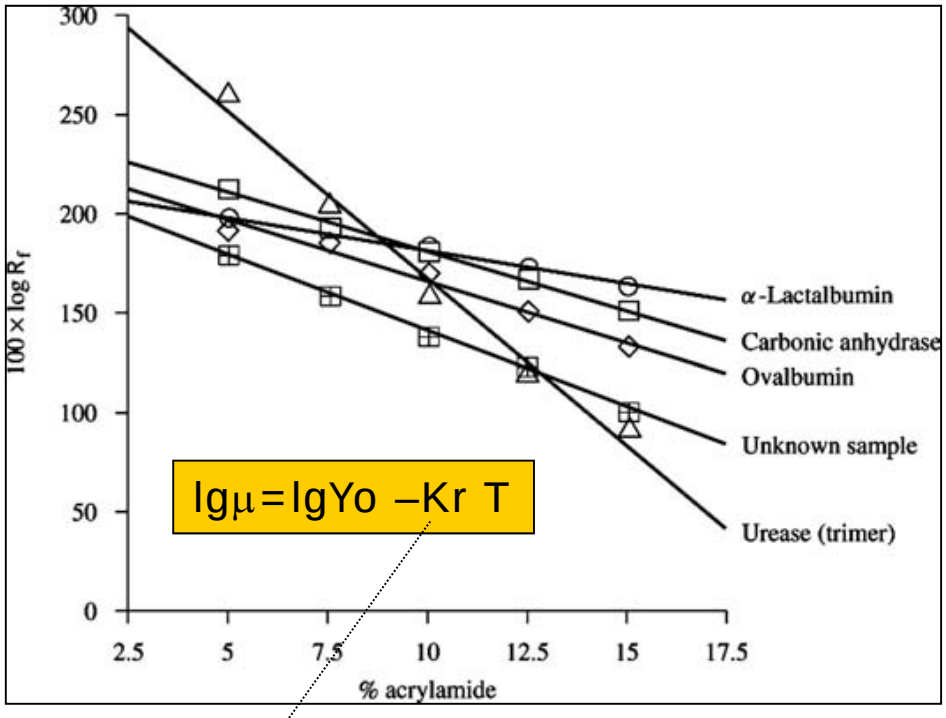
Azul de bromofenol
+ glicerina (densidad)







ELECTROFORESIS NO DESNATURALIZANTE



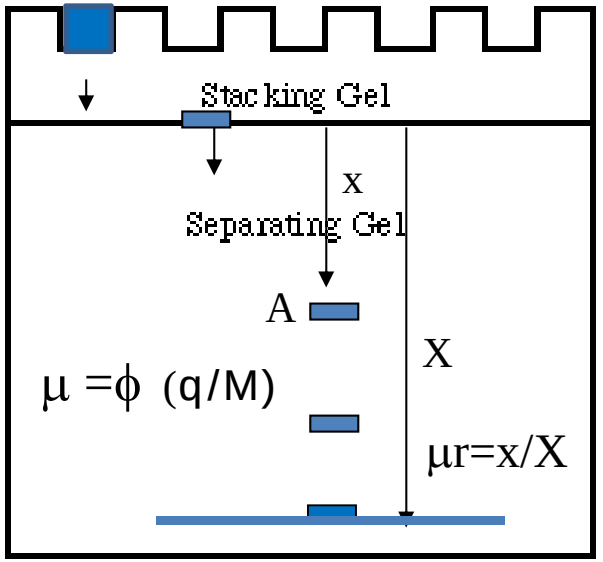
$lg\mu = lgYo - Kr\ T$

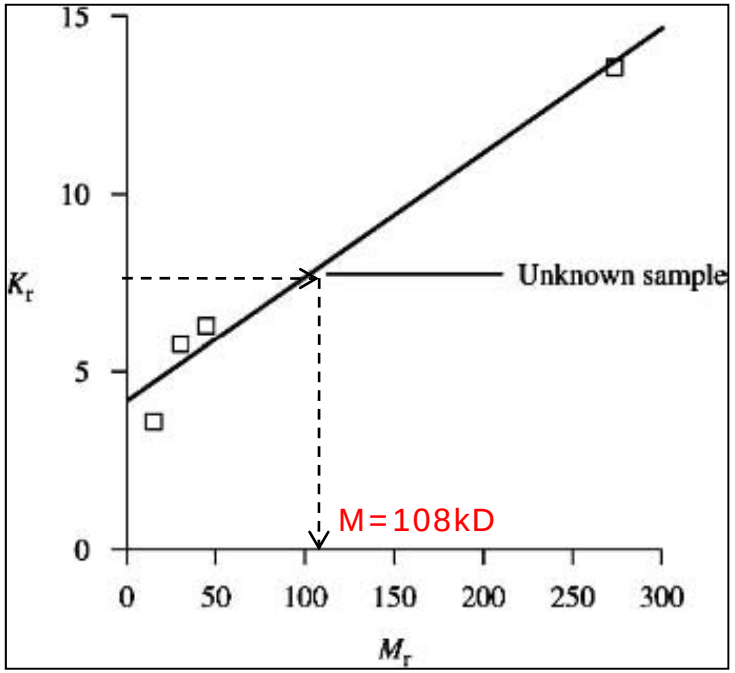
A más % de acrilamida, para una proteína menos movilidad, menos Rf

Representaciones de Ferguson

Kr (coeficiente de retardo)= ϕ (radio molecular)

14,2kD
29kD
45kD
¿?
272kD

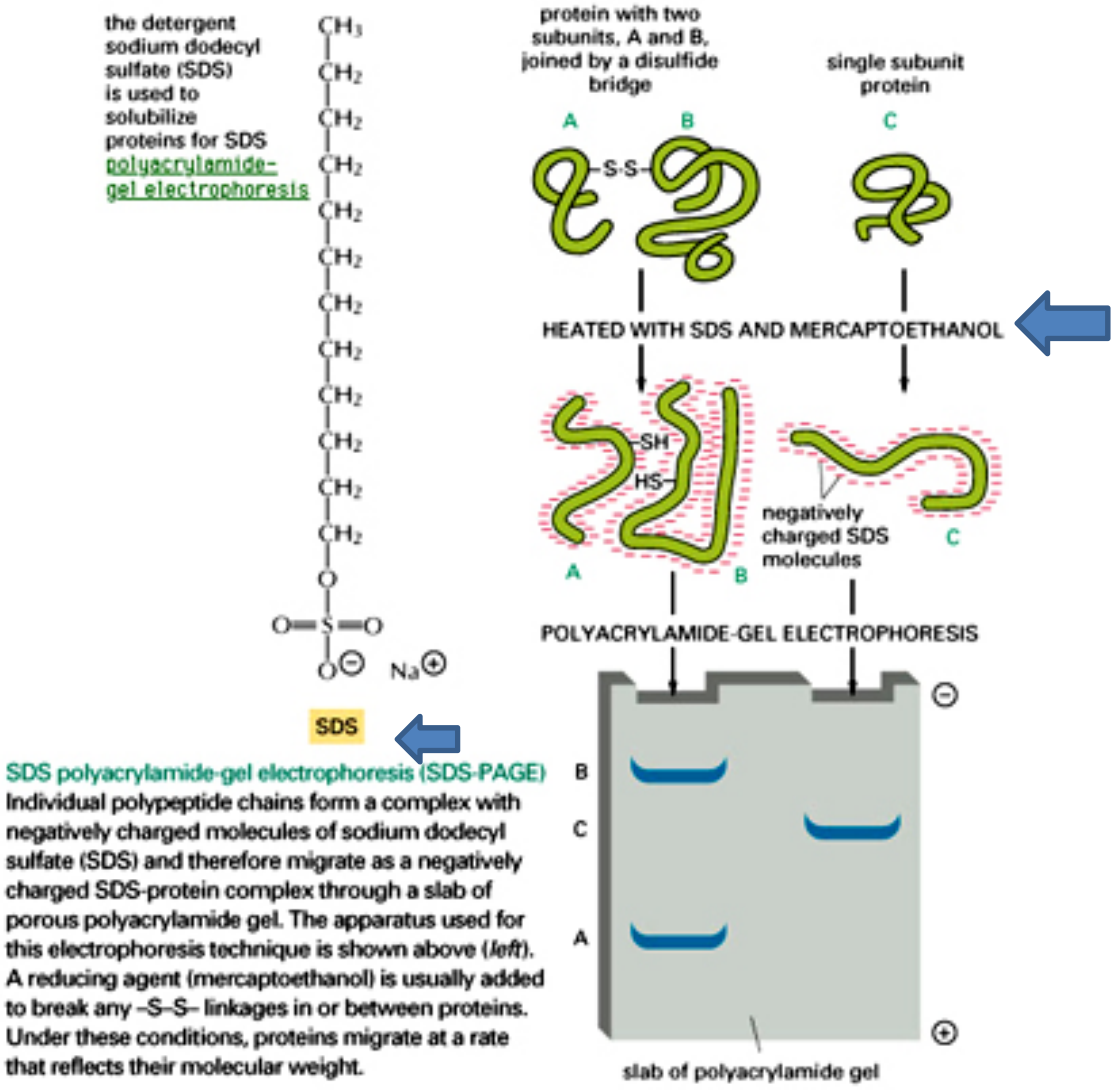


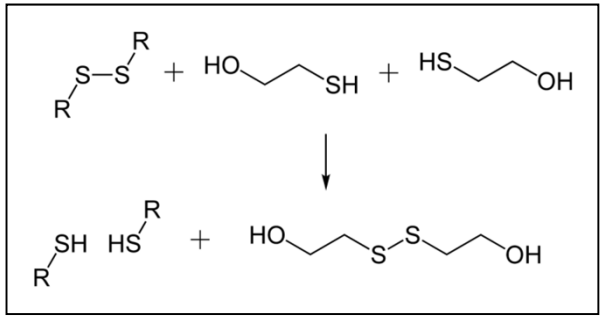


ELECTROFORESIS DESNATURALIZANTE
SDS-PAGE

$\mu = \phi$ Q/M ➔no desnaturalizante

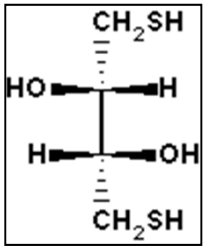
$\mu = \phi$ 1 / M ➔con SDS



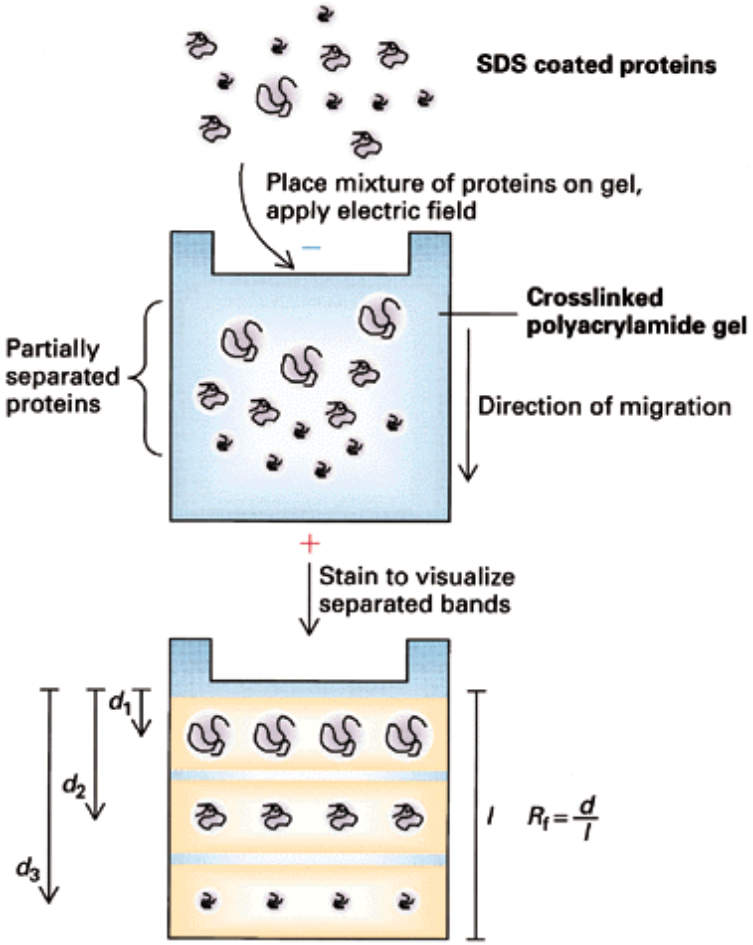


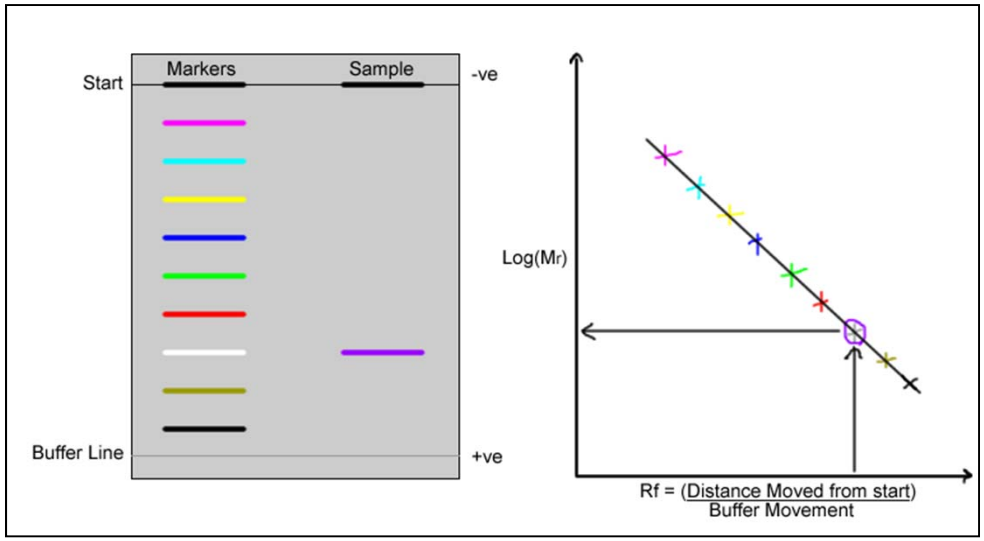
Beta.mercaptoetanol

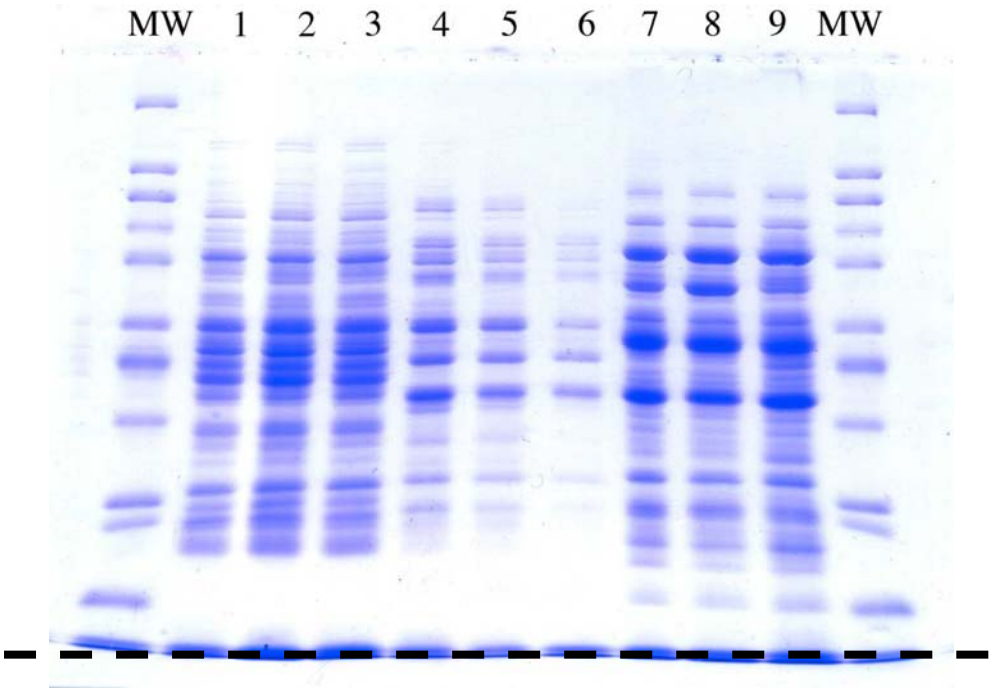
Ditiotreitol
DTT



AGENTES REDUCTORES


SDS coated proteins
Place mixture of proteins on gel, apply electric field
Crosslinked polyacrylamide gel
Partially separated proteins
Direction of migration
Stain to visualize separated bands
d_1
d_2
d_3
l
$R_f = \frac{d}{l}$


Markers
Sample
Start
-ve
Buffer Line
+ve
Log(Mr)
Rf = (Distance Moved from start)
Buffer Movement


MW 1 2 3 4 5 6 7 8 9 MW

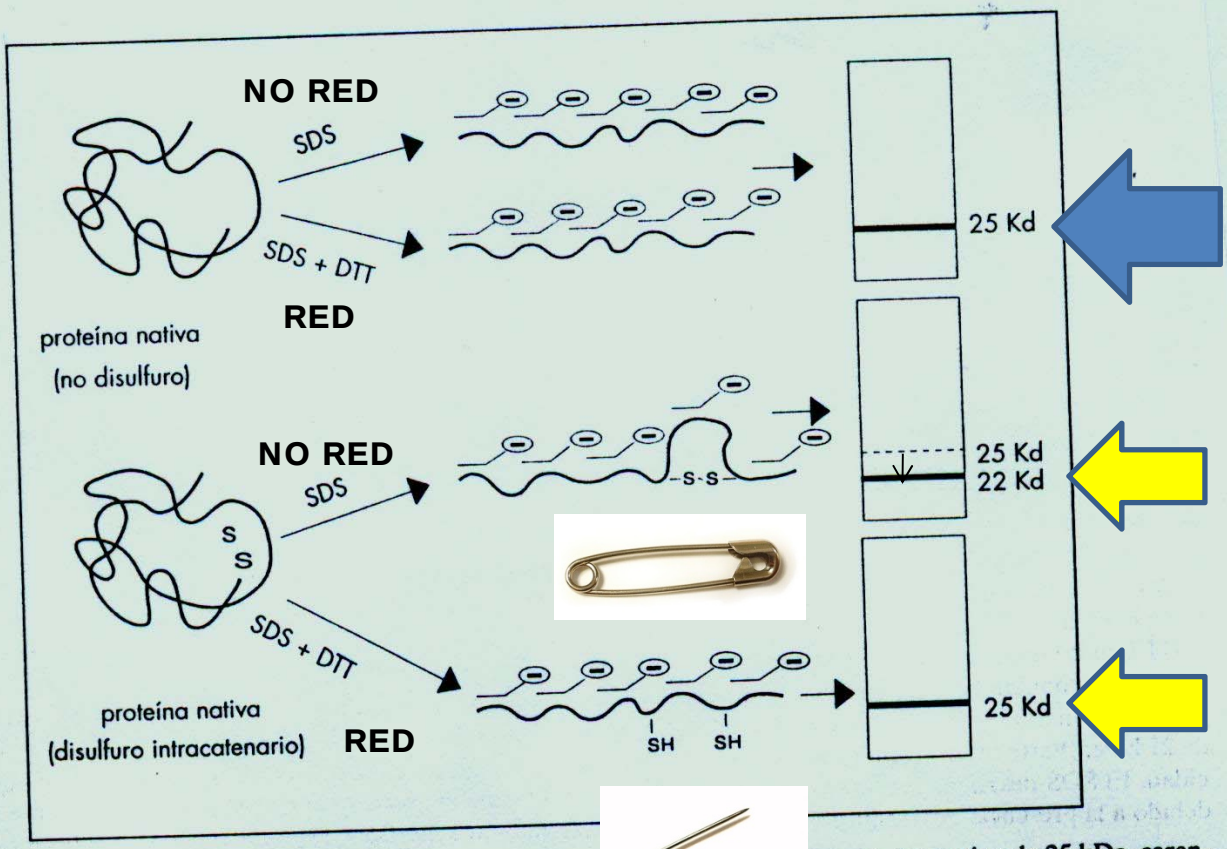


FIGURA 5.27. Efecto del SDS y el DTT en la *Ur* de proteínas m…a proteína nativa de 25 kDa, caren-
te de puentes disulfuro, el tratamiento con SDS (línea quebr…) o SDS + DTT, producen idéntico
resultado: una banda en el gel (rectángulo vertical) en la posici…te a la M de la proteína, 25 kDa. El
SDS despliega la proteína convirtiéndola en una molécula aproximadamente lineal. La proteína con un disulfuro
intracatenario, en presencia de SDS, se desnaturaliza parcialmente, manteniendo sin desplegar la zona que abarca el
disulfuro. La proteína se comporta como si fuera de menor tamaño, apareciendo en la posición correspondiente a un
valor de M de 22 kDa. Cuanto mayor sea el segmento englobado por el disulfuro (cuanto más distantes estén en la
estructura primaria las Cys que lo forman), mayor es el cambio observado en la posición de la banda. En trazo dis-
continuo se señala la posición de 25 kDa. El tratamiento con SDS + DTT rompe el enlace disulfuro y permite el des-
pliegue total de la proteína, que aparece en la posición correspondiente a 25 KDa. El cambio de posición de la
banda, con ambos tratamientos, permite identificar la presencia de uno (o más) puentes disulfuro intracatenarios.

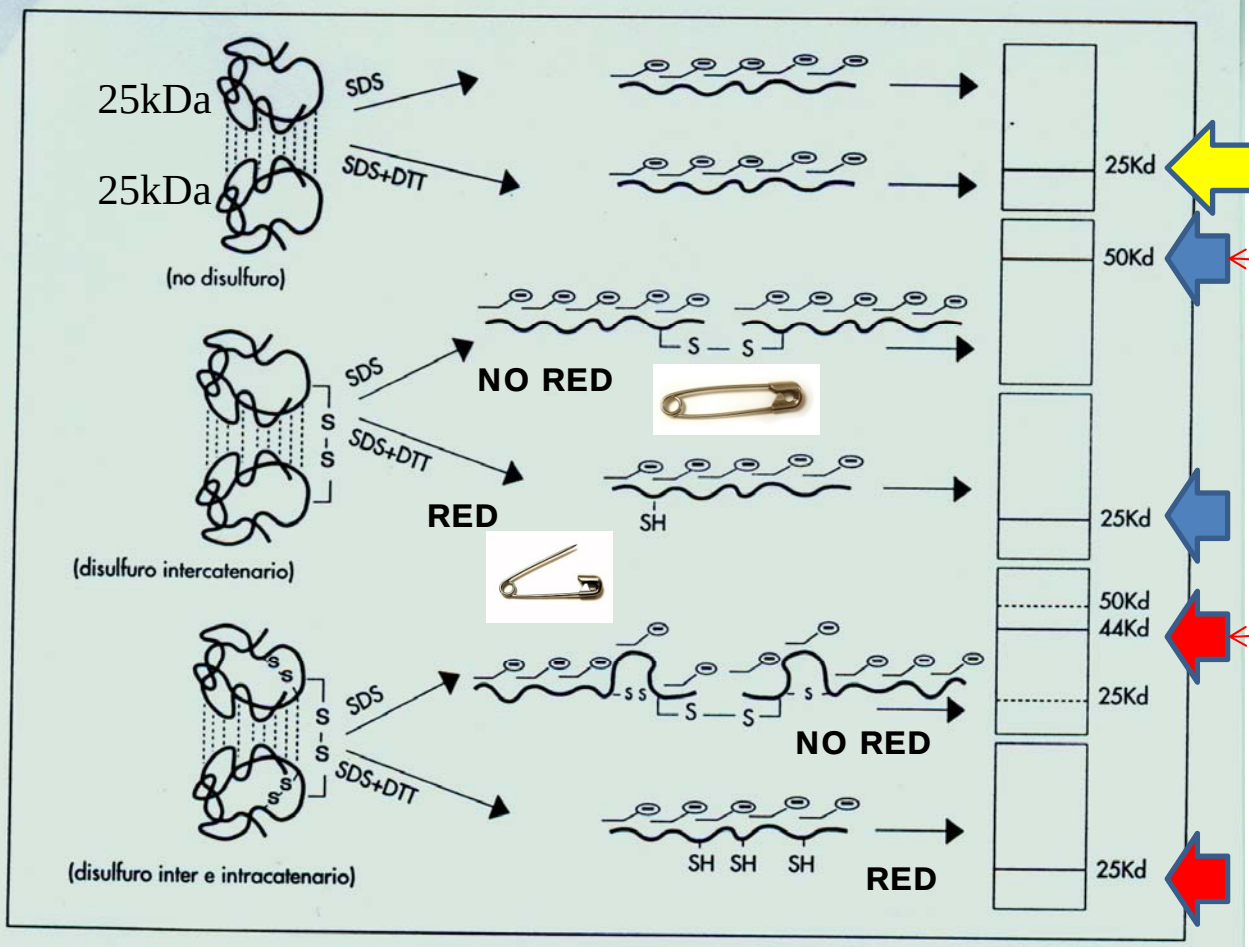
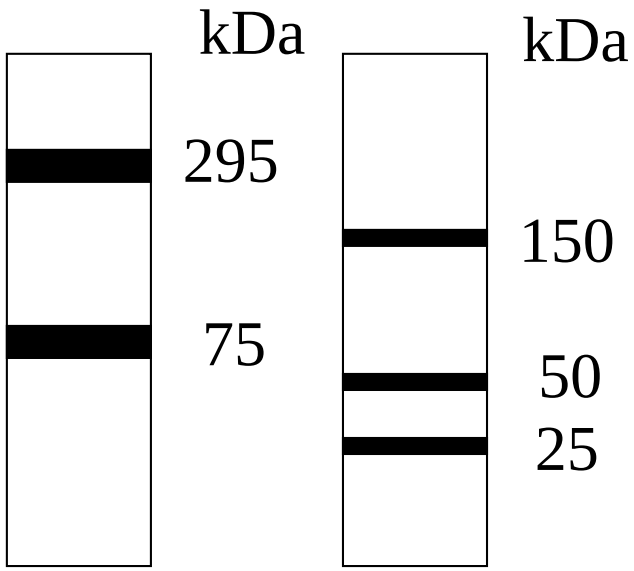
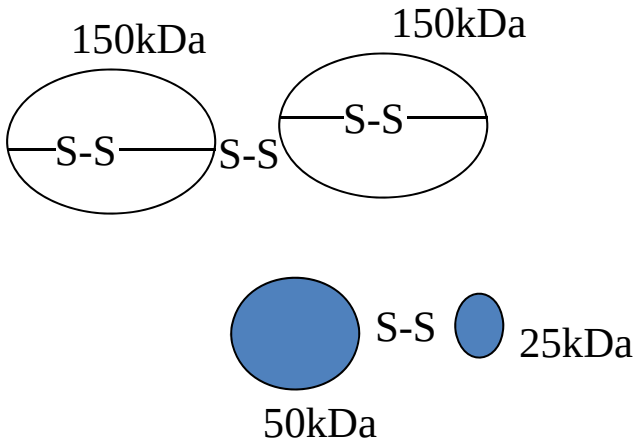


FIGURA 5.28. Efecto del SDS y del DTT en la *Ur* de proteínas oligoméricas. (Parte superior): representación de una proteína dimérica, de 25 kDa cada subunidad. La adición de SDS (línea quebrada con el signo –) separa sus subunidades, suprimiendo las fuerzas no covalentes (en punteado) entre ellas. Sobre cada subunidad, el efecto es el descrito en la Figura 5.27. En el gel se observa una sola banda en la posición de 25 KDa. El resultado es idéntico con SDS + DTT, al no haber puentes disulfuro. (Parte central): dímero con un puente disulfuro intercatenario. El SDS despliega las subunidades que, mantenidas por el disulfuro, aparecen en el gel como una banda de unos 50 kDa. El tratamiento con SDS + DTT reduce el disulfuro y despliega cada subunidad (idénticas), obteniéndose una sola banda de 25 kDa. (Parte inferior): la proteína tiene, en este caso, además, un enlace disulfuro intracatenario en cada subunidad. El SDS despliega parcialmente cada subunidad. La banda aparece en la posición correspondiente a 44 kDa debido a la presencia del disulfuro intercatenario. El tratamiento con SDS + DTT destruye ambos tipos de disulfuro, apareciendo, nuevamente, una única banda a 25 kDa.

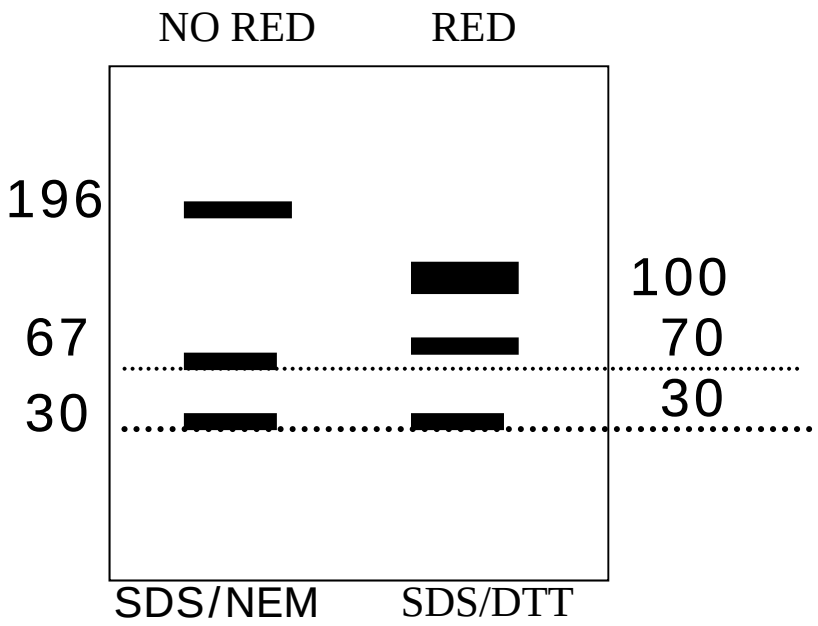


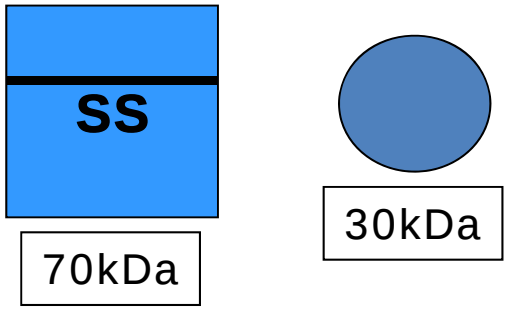
A
SDS

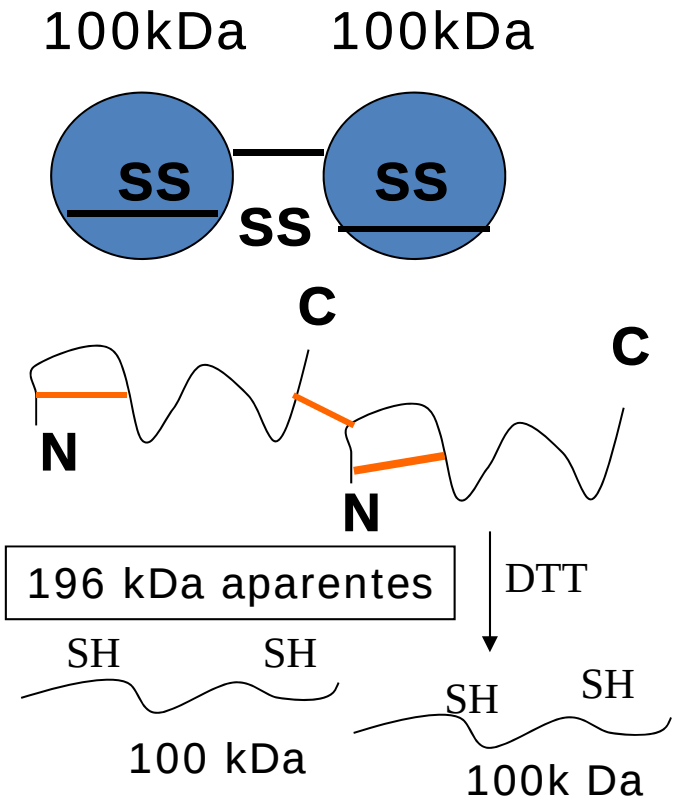
B
SDS+DTT



En una muestra de proteinas purificada se obtienen los resultados esquematizados tras la electroforesis en SDS-poliacrilamida. En la condición 1 no reductora: 3 bandas de 196, 67 y 30 kDa y en la condición 2 en condiciones reductoras, en presencia de ditiotreitol (DTT) 3 bandas de 100, 70 y 30 kDa
¿Qué caracteristicas podrían deducirse sobre las proteinas de la mezcla?







SOLUCIONES PARA ELECTROFORESIS MONODIMENSIONAL 2 PLACAS

	7.5%	10%	12.5%	15%
AGUA DESTILADA	21	17.4	14	10.4
LOWER BUFFER	10.5	10.5	10.5	10.5
ACRILAMIDA (30:1)	10.5	13.8	17.3	20.8

UREA añadir 15,12g y llevar con agua hasta 42ml

TEMED..... 22µl
PERSULFATO..... 137µl

RUNNING BUFFER

TRIS .....6 g
GLICINA .....28.75 g
AGUA DESTILADA... 2 litros
SDS .....2 g

UPPER BUFFER

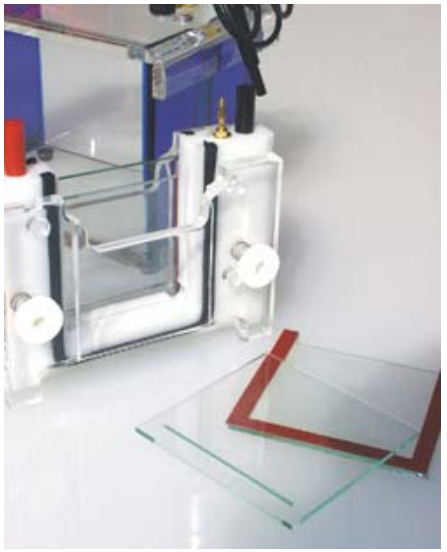
TRIS .....60.54 g (0.5M) pH 6.8
AGUA DESTILADA...1 litro
SDS.....4 g

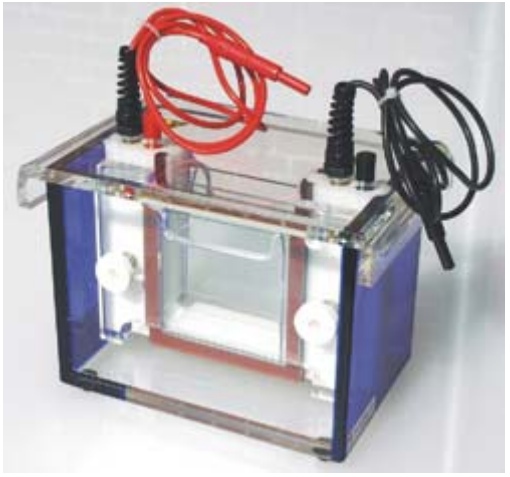
LOWER BUFFER

TRIS.....181.1 g (1.5M) pH 8.8
AGUA DESTILADA.. 1 litro
SDS.....4 g

WESTERN BLOT BUFFER

TRIS.....9 g
GLICINA.....42 g
AGUA DESTILADA.....2.5 litros
METANOL.....500 ml





p5.pdf - Adobe Reader

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Práctica 5: Electroforesis en geles de poliacrilamida (PAGE).

1.- INTRODUCCIÓN

Muchas moléculas importantes en Bioquímica, tales como aminoácidos, péptidos, proteínas y ácidos nucleicos, poseen grupos ionizables que en disolución se encuentran en forma de especies cargadas eléctricamente (negativa o positivamente). Las moléculas que tengan cargas similares poseerán diferentes relaciones carga/masa (q/m) debido a las inherentes diferencias de peso molecular. Estas diferencias constituyen la base para la migración diferencial de dichas moléculas cargadas, cuando se someten a la acción de un campo eléctrico.

La **electroforesis en geles de poliacrilamida (PAGE)** se utiliza mayoritariamente para la separación de proteínas, aunque también puede ser útil para ácidos nucleicos. Se preparan de modo que sus poros sean de un tamaño comparable al de las proteínas, de manera que produzcan un efecto de tamizado molecular; la separación electroforética depende entonces de la densidad de carga de las moléculas y de su tamaño, por lo que dos proteínas con idéntica densidad de carga, pero de tamaño diferente pueden ser separadas, ya que el

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Gel separador 12 % **1 gel**

Agua destilada➔ 1,7 ml

Tampón Tris 1,5 M; pH=8,8 ➔1,25 ml

Acrilamida:Bis acrilamida 30:0,8 ➔ 2 ml

SDS 10% ➔ 50 µl

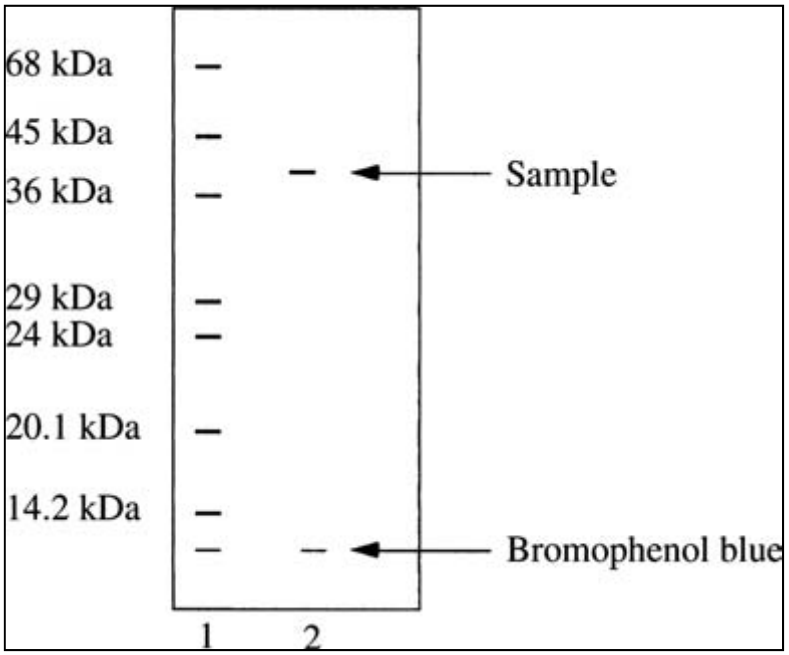
Gel concentrador 4 % **1 gel**

Agua destilada ➔ 1,5 ml

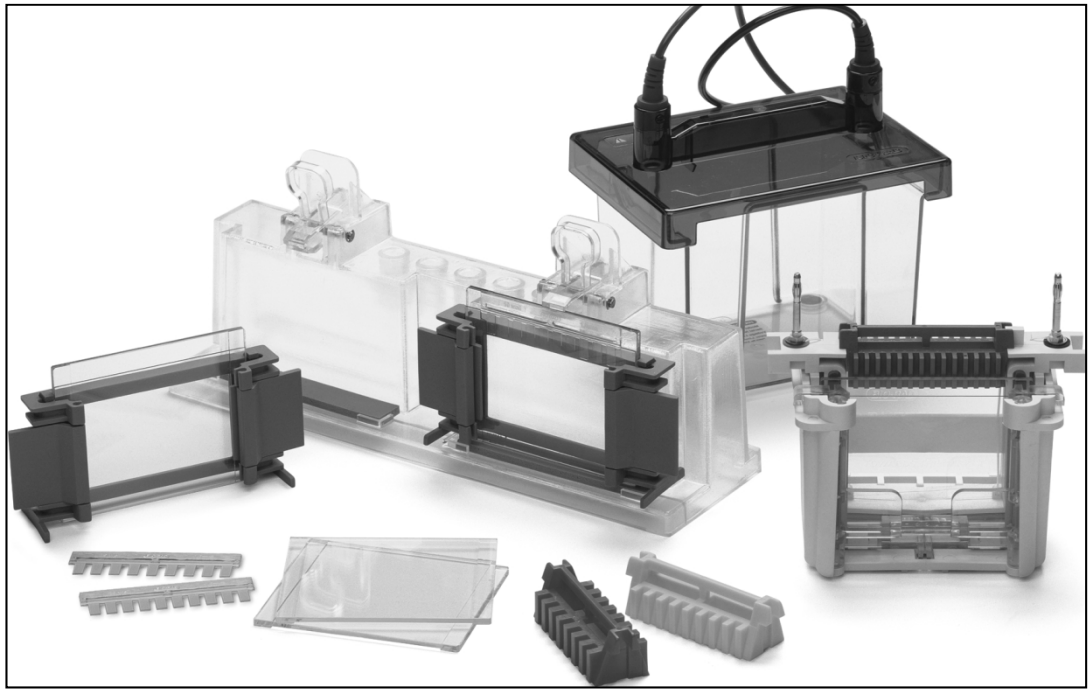
Tris-HCl 0,5 M; pH=6,8 ➔ 0,625 ml

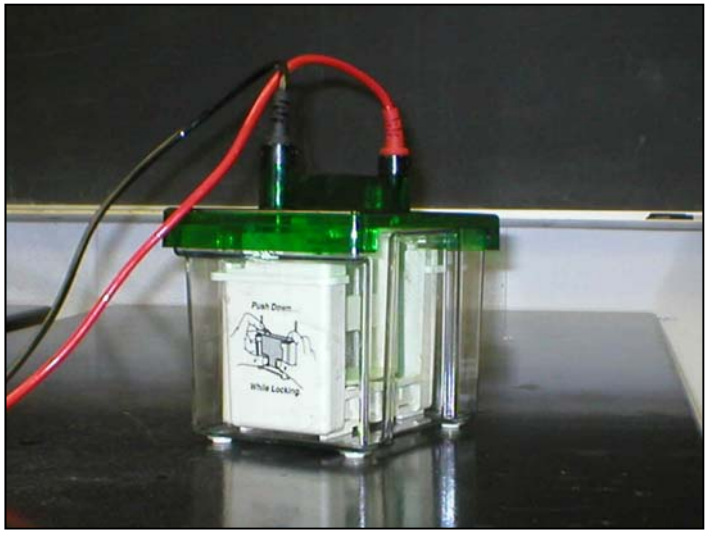
Acrilamida:Bis acrilamida 30:0,8 ➔0,325 ml

SDS 10% ➔25 µl



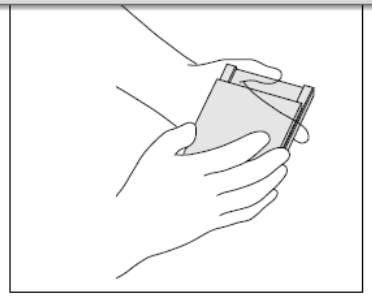
Tampón para muestras	**Reductor (5X)**	**No reductor (5X)**
H2O	-------	5 ml
Tris-HCl 1,5 M pH 6,8	4 ml	4 ml
Glicerol 100 %	10 ml	10 ml
SDS	2 g	2 g
2-Mercaptoetanol	5 ml	---------
Azul de Bromofenol 0.1%	1 ml	1 ml



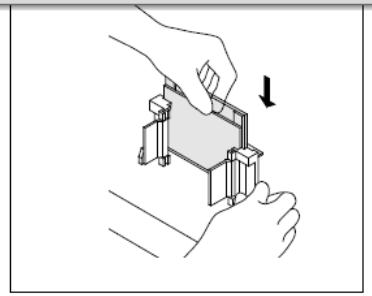


http://www.youtube.com/watch?v=pnBZeL8nFEo

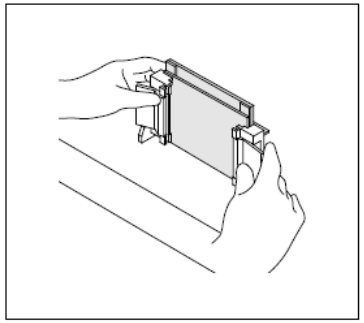
http://www.youtube.com/watch?v=gcJg_GtLgt8&feature=related



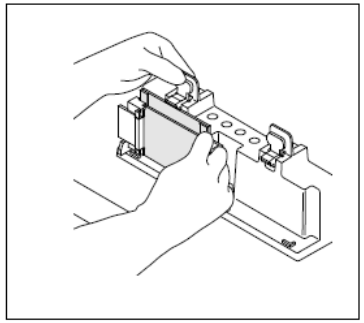
4a. Place a Short Plate on top of the Spacer Plate.



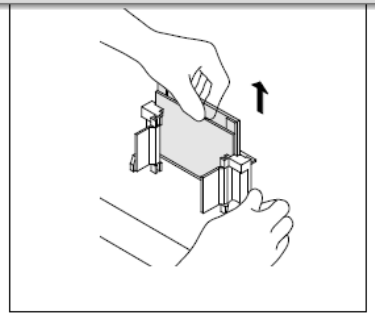
4b. Slide the two plates into the Casting Frame keeping the Short Plate facing front.



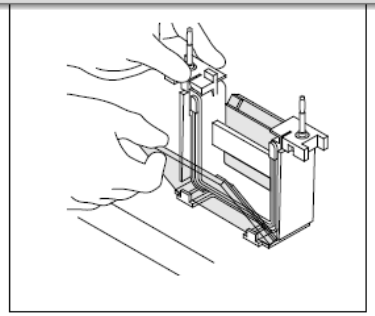
4c. Lock the pressure cams to secure



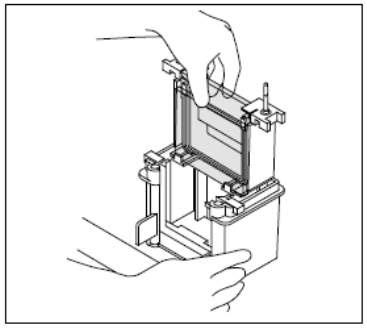
4d. Secure the Casting Frame in the Casting



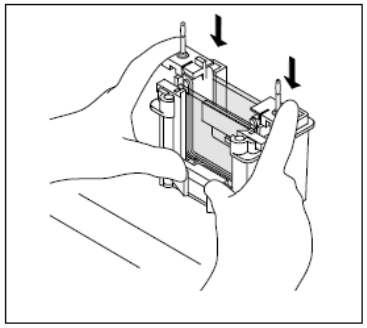
5a. Remove the Gel Cassette Sandwich from the Casting Frame.



5b. Place Gel Cassette Sandwich into the Electrode Assembly with the Short Plate facing Inward.



5c. Slide Gel Cassette Sandwiches and



5d. Press down on the Electrode Assembly

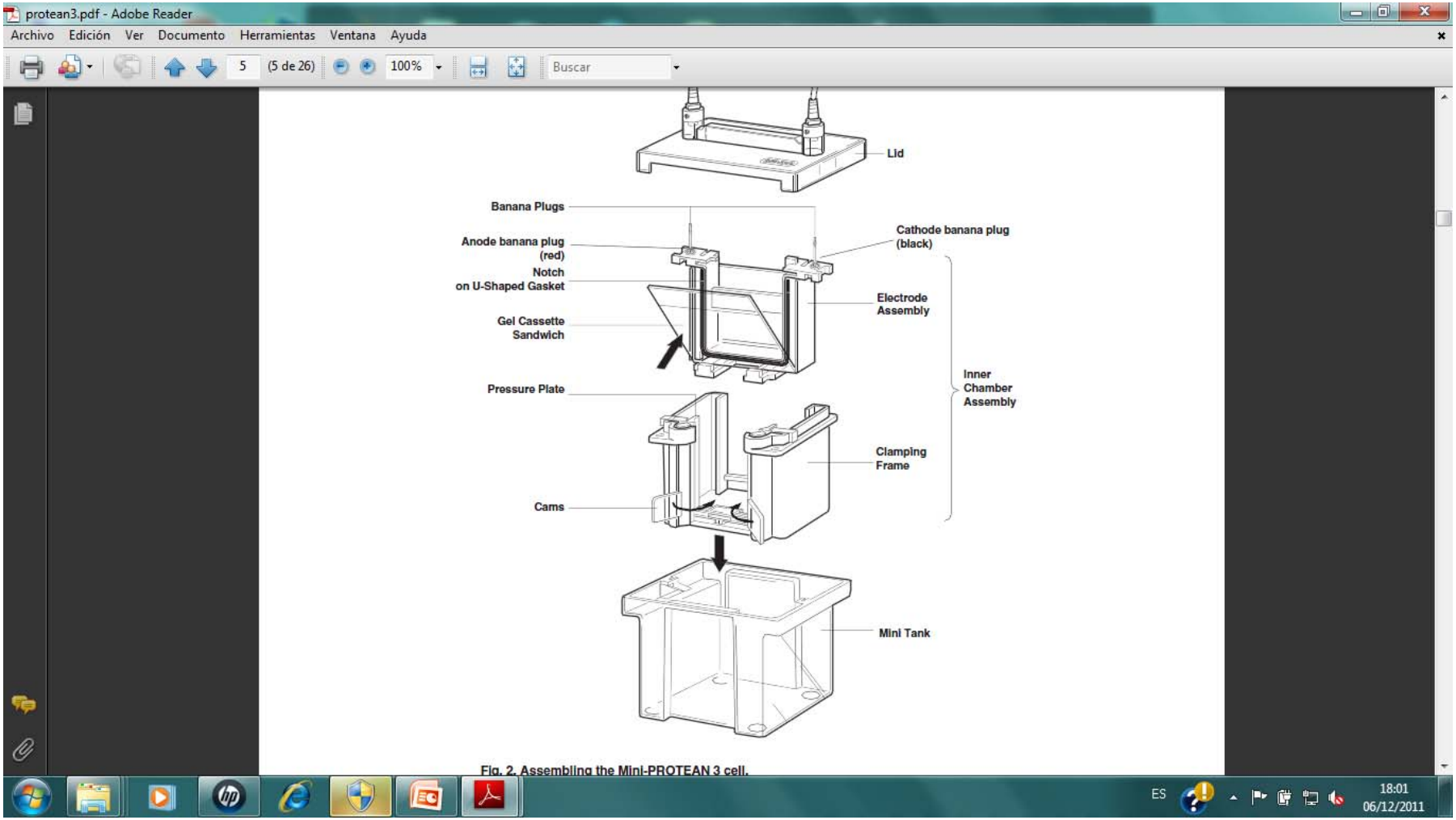
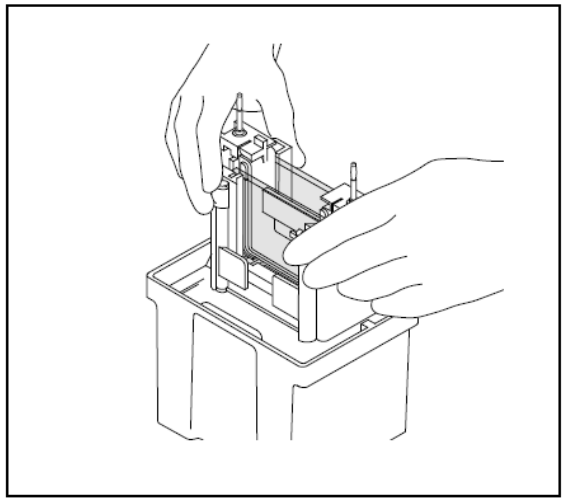


Fig. 2. Assembling the Mini-PROTEAN 3 cell.

Frame. Clamping Frame.

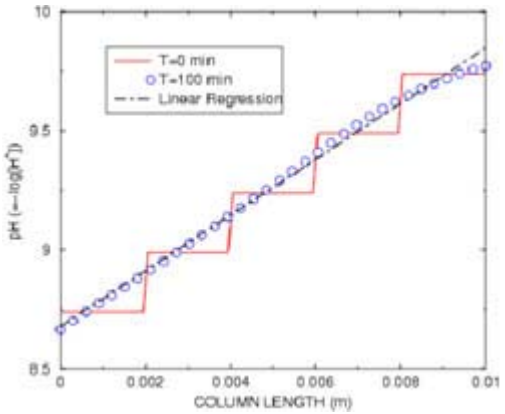


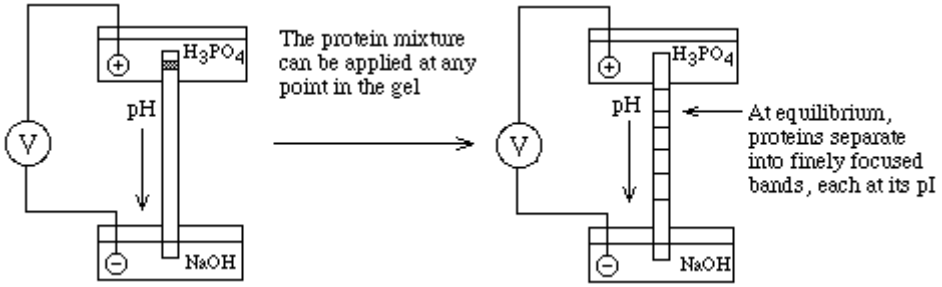
5e. Lower the Inner Chamber into the Mini Tank.

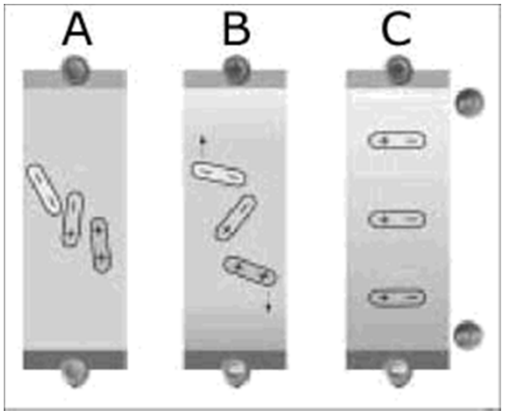
Fig. 5. Mini-PROTEAN 3 assembly.

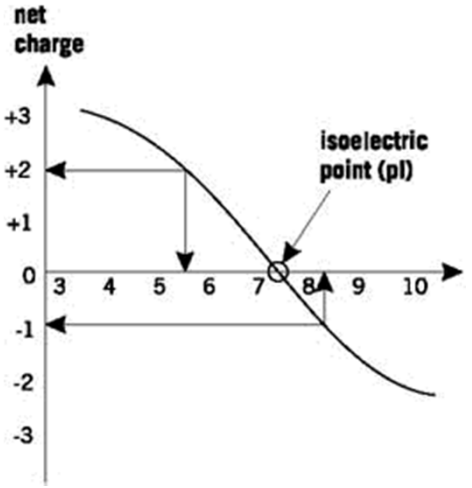
ELECTROENFOQUE :IEF O ISOELECTRIC FOCUSING

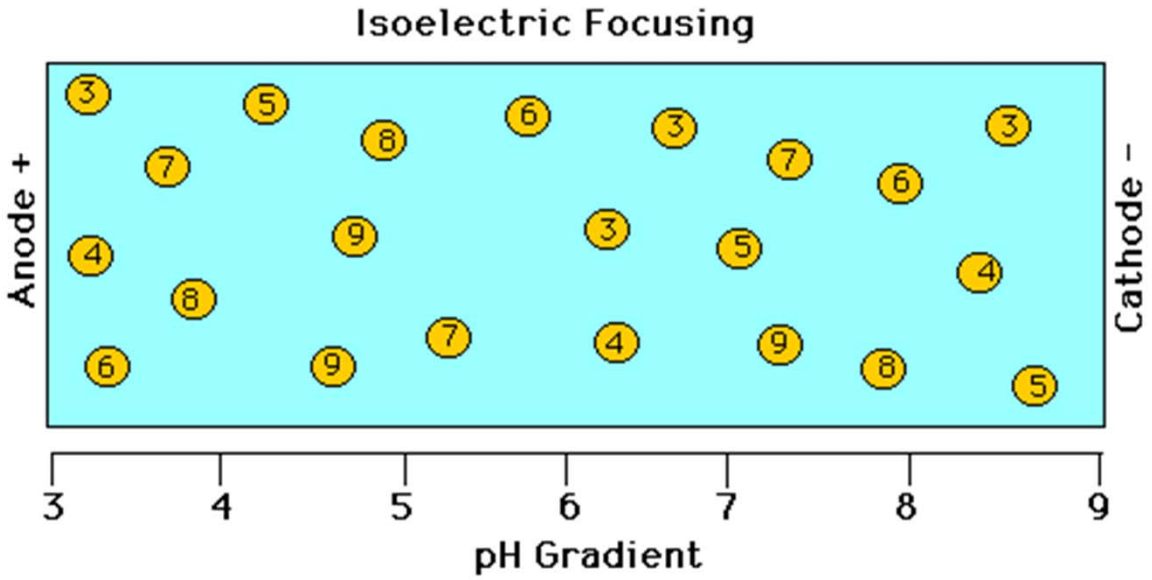
Migración de moléculas a través de un campo eléctrico en el que existe un gradiente de pH



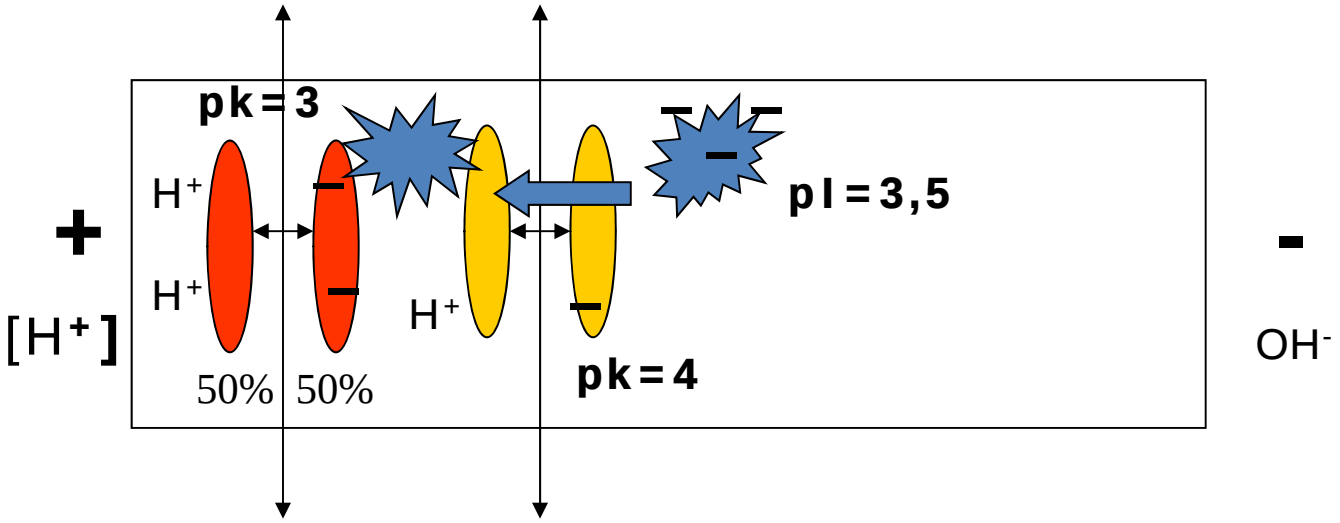


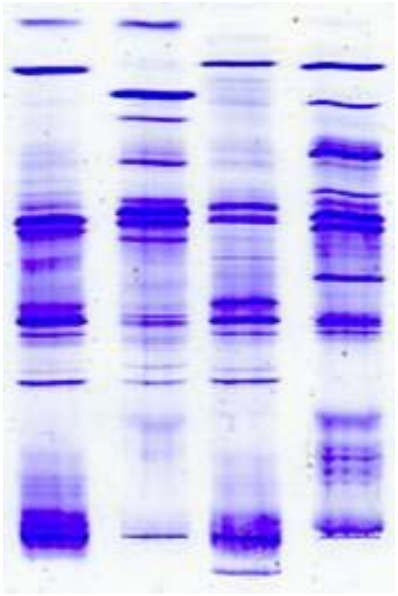





Isoelectric Focusing
Anode +
Cathode –
3
4
5
6
7
8
9
pH Gradient

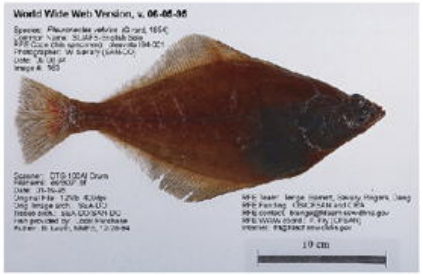

SERVA
SERVALYT

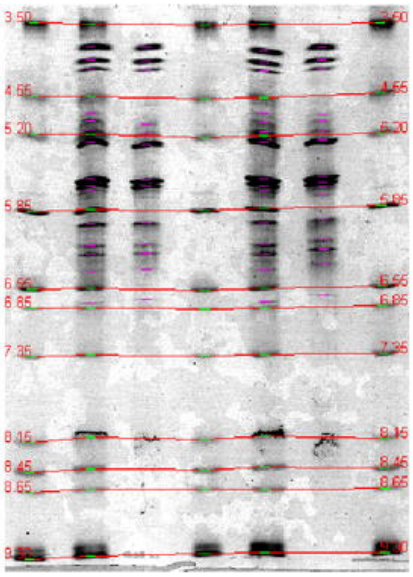

+
[H+]
pk = 3
H+
H+
50%
50%
H+
pk = 4
pI = 3,5
-
OH-

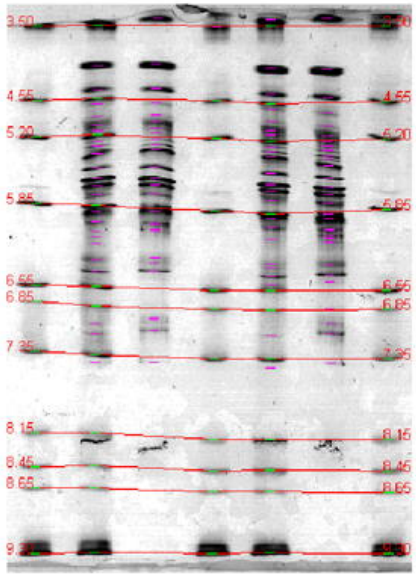


ISOENZIMAS
Mismo PM
Distinto PI

Calibrated Gel Comparison





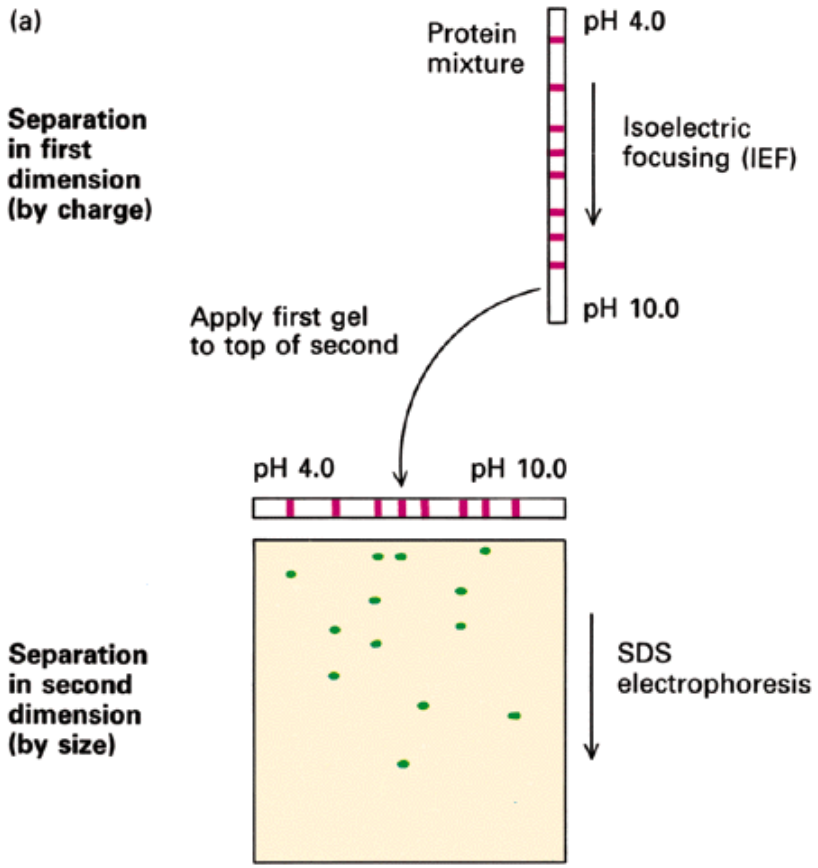


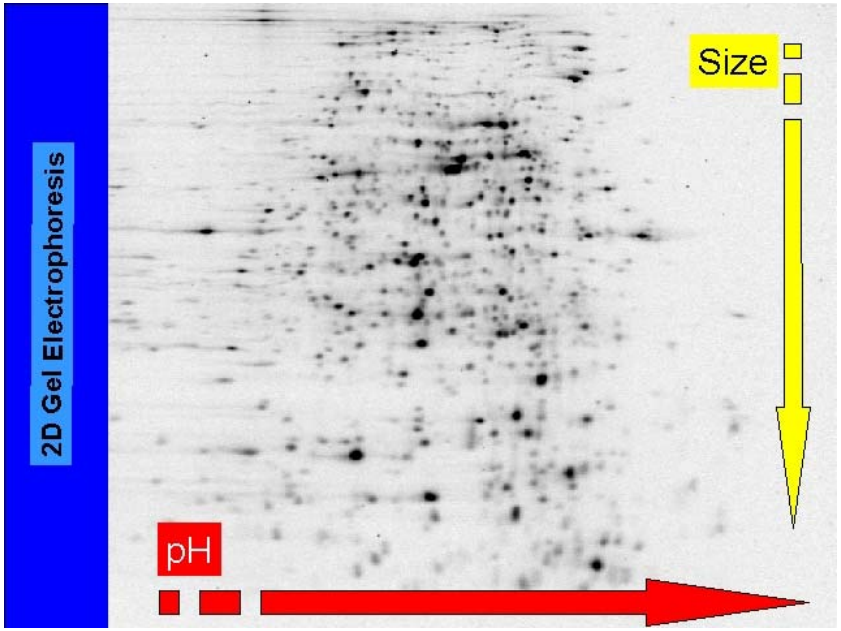
Arrowtooth Flounder
athestom 002 #1
081393 gel #3

English Sole
pleuvetu 002 #1
081793 gel #2

RFE Team: Tenge, Barnett, Savary, Rogers, Fry, Dang
RFE Contact: btenge@fdaem.ssw.dhhs.gov
frf@fdacf.ssw.dhhs.gov
ndang@fdaem.ssw.dhhs.gov

2D PAGE: ELECTROFORESIS BIDIMENSIONAL = IEF + PAGE





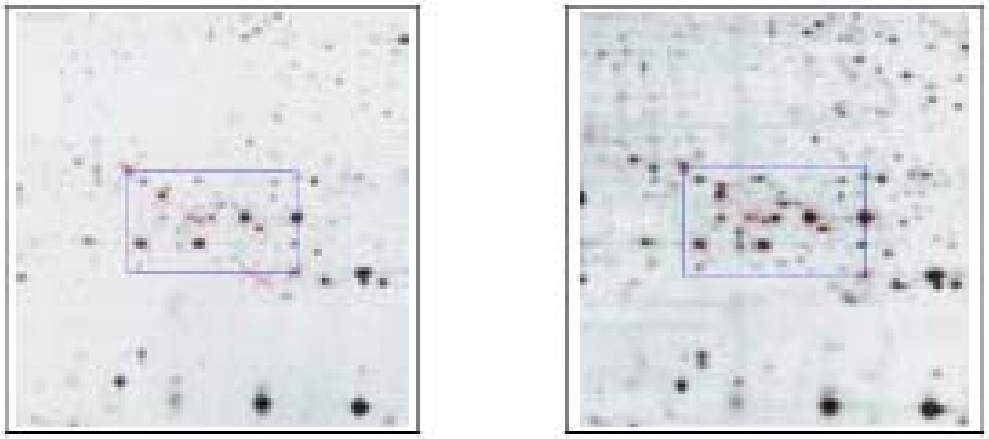
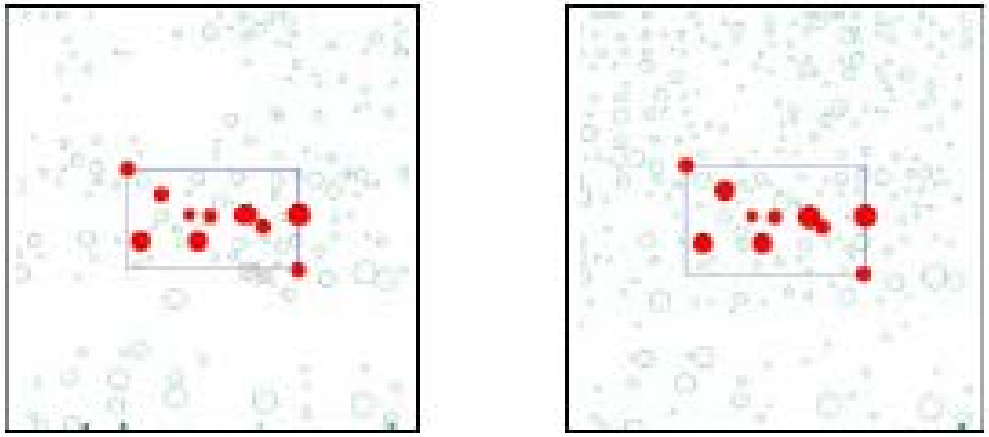
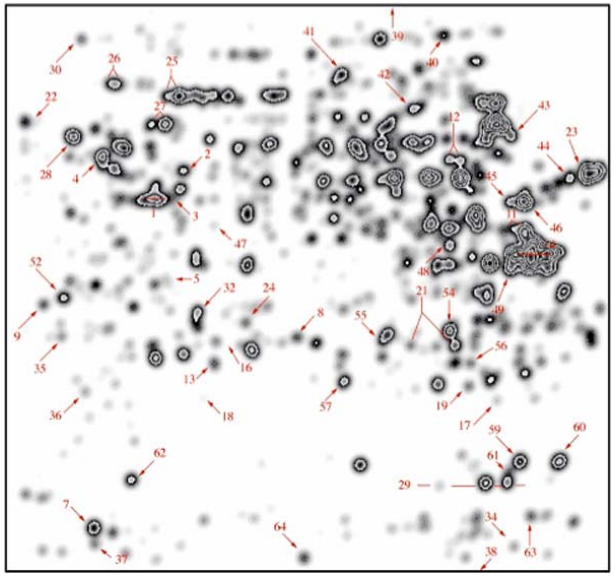
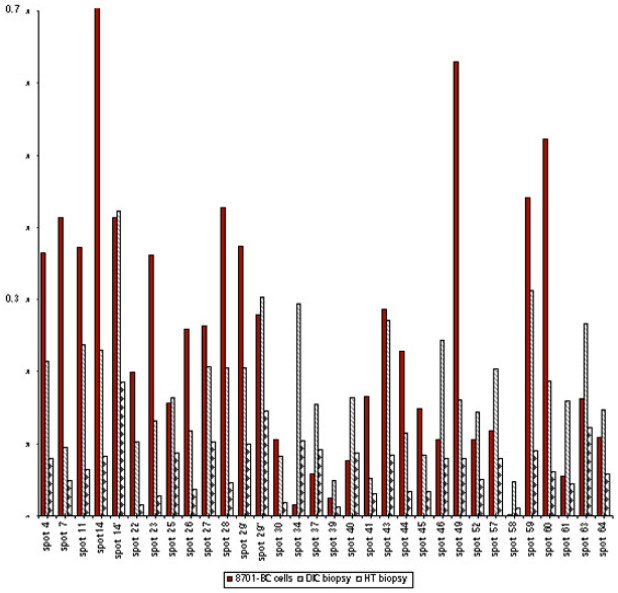
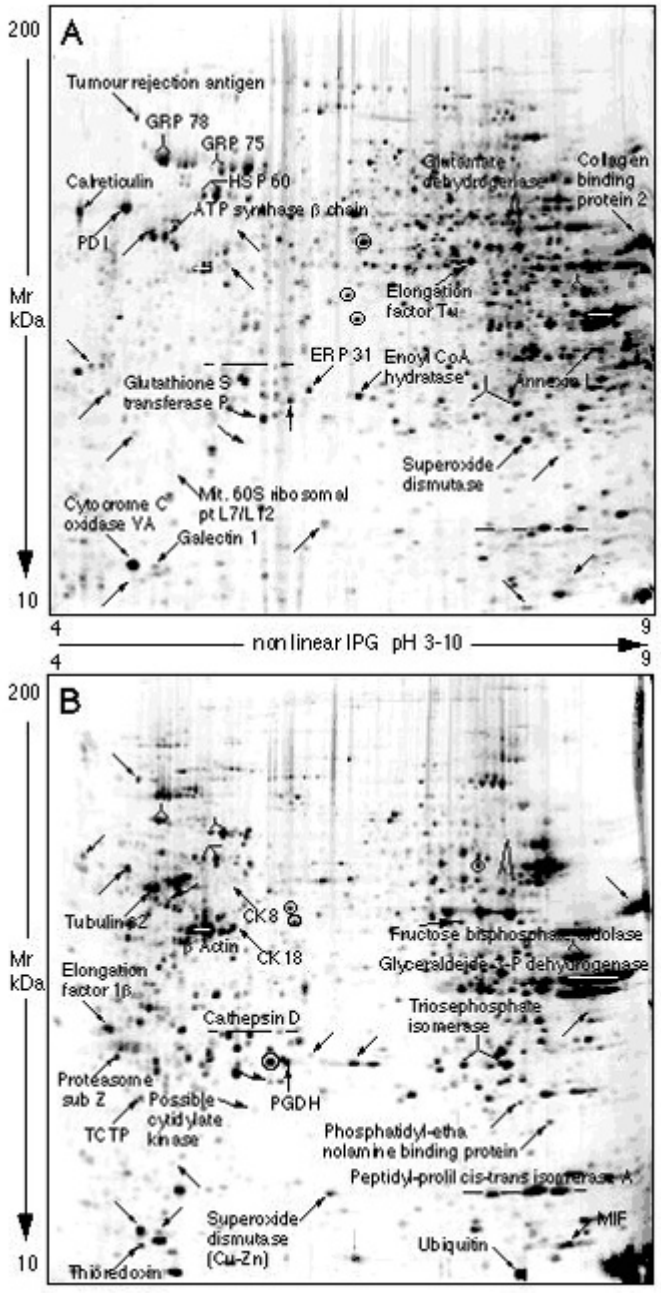


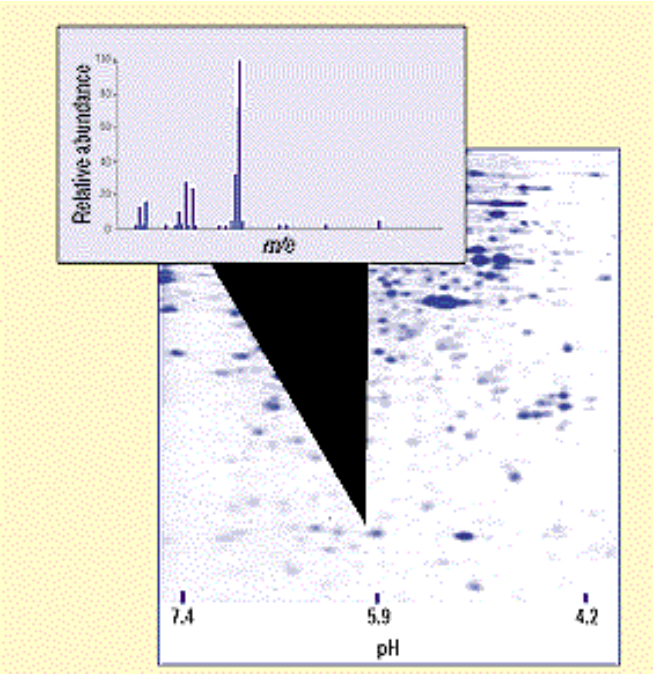
Figure 3: Detailed local images of Fig.2, a selected pattern on the left side and a partial matching

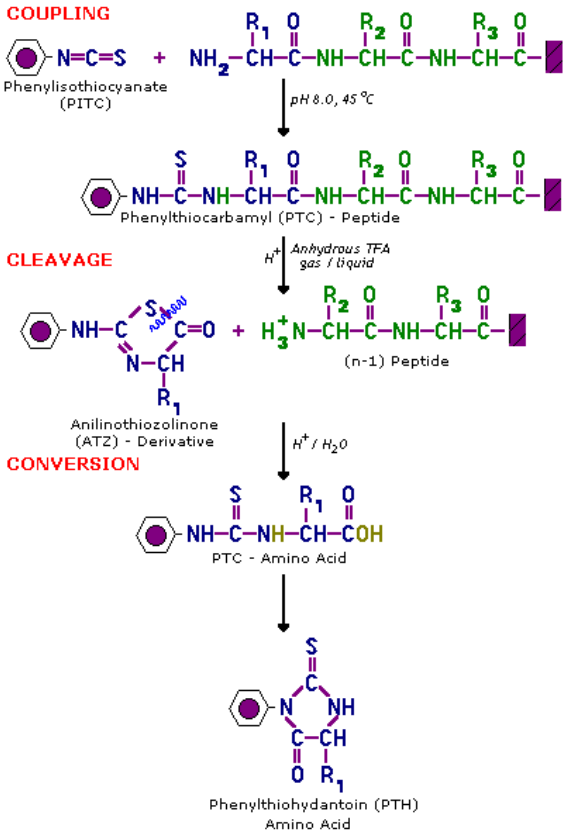


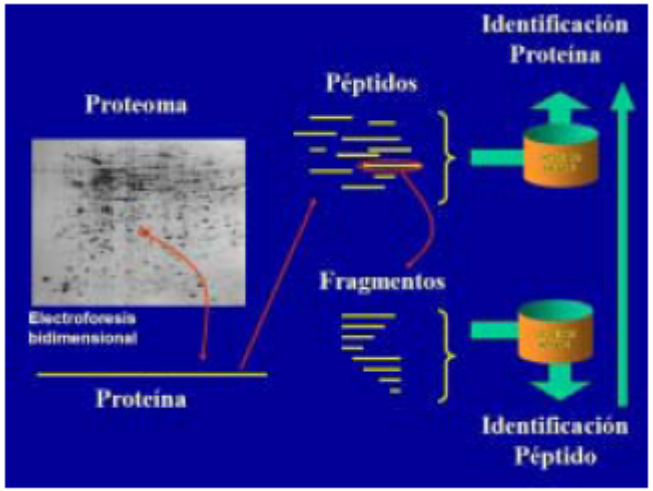



0.7
0.3
spot 4
spot 7
spot 11
spot14
spot 14
spot 22
spot 23
spot 25
spot 26
spot 27
spot 28
spot 29
spot 29'
spot 30
spot 34
spot 37
spot 39
spot 40
spot 41
spot 43
spot 44
spot 45
spot 48
spot 49
spot 52
spot 57
spot 58
spot 59
spot 60
spot 61
spot 63
spot 64
8701-BC cells
DIC biopsy
HT biopsy


A
200
Mr
kDa
10
Tumour rejection antigen
GRP 78
GRP 75
HSP 60
Calreticulin
ATP synthase β chain
PDI
Glutamate dehydrogenase
Collagen binding protein 2
Elongation factor Tu
ERP 31
Enoyl CoA hydratase
Annexin I
Glutathione S transferase P
Superoxide dismutase
Cytocrome C oxddase VA
Mt. 60S ribosomal pt L7/L12
Galectin 1
4
9
non linear IPG pH 3-10
4
9
B
200
Mr
kDa
10
Tubulin α2
CK8
β Actin
CK18
Fructose bisphosphate aldolase
Glyceraldejde-3-P dehydrogenase
Elongation factor 1β
Cathepsin D
Triosephosphate isomerase
Proteasome sub Z
Possible cytidylate kinase
PGDH
TCTP
Phosphatidyl-etha nolamine binding protein
Peptidyl-prolil cis-trans isomerase A
Superoxide dismutase (Cu-Zn)
MIF
Ubiquitin
Thioredoxin


Relative abundance
m/z
7.4
5.9
4.2
pH


COUPLING
Phenylisothiocyanate
(PITC)
pH 8.0, 45 °C
Phenylthiocarbamyl (PTC) - Peptide
CLEAVAGE
Anhydrous TFA
gas / liquid
(n-1) Peptide
Anilinothiozolinone
(ATZ) - Derivative
CONVERSION
PTC - Amino Acid
Phenylthiohydantoin (PTH)
Amino Acid

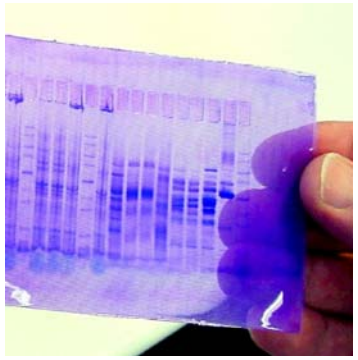

Identificación
Proteína
Proteoma
Péptidos
Fragmentos
Electroforesis
bidimensional
Proteína
Identificación
Péptido

DETECCION Y ANALISIS DE PROTEINAS TRAS ELECTROFORESIS

1.-PREPARACION DEL GEL
2.-APLICACIÓN DE LA MUESTRA
3.-CORRER EL GEL (RUNNING)
4.-DETECCION DE PROTEINAS
5.-SECAR Y PROCESAR: DENSITOMETRIA, PROTEOMICA

TINCION: DETECCION POR COLORANTES O SUSTRATOS QUIMICOS
AUTORADIOGRAFIA: DETECCION MEDIANTE MARCAJE CON RADIOISOTOPOS
INMUNODETECCION: DETECCIÓN ESPECIFICA CON ANTICUERPOS

TINCION RAPIDA: COOMASSIE (microgramos)
TINCION SENSIBLE: PLATA (nanogramos)
TINCION PROTEOMICA: sypro ruby fluorescente



ELECTROPHORESIS

Protein Staining Reagents

To meet the great diversity of protein analysis needs, Sigma offers a wide selection of protein visualization (staining) reagents. EZBlue™ and ProteoSilver™, designed specifically for proteomics, also perform impressively in traditional PAGE formats.

Protein Dyes and Stains Selection Chart

Optimize your protein detection by choosing the best reagent for your application.

✓ Good Choice

★ BEST Choice

		Alcian Blue 8GX — A 3157	Amido Black Staining Solution — A 1383	Brilliant Blue G Concentrate — B 1322	Brilliant Blue R Staining Solution — B 6529	Coomassie™ Violet R 200 — 27817	Eosin Y — 45235	EZBlue™ — G 1041	Fast Green FCF — F 7252	Fixing Solution — F 7264	Fluorescamine — F 9015	GlycoProbe H — HT 0008	GlycoProtein Detection Kit (Colorimetric) — GKCOLPRO	Gold Solution, Colloidal — 50226	O-Red O — O 0125	Ponceau S Solution — P 3100	ProteoSilver™ — PROT-SIL1	ProteoSilver Plus — PROT-SIL2	Reversible Protein Detection Kit — R PROT	Sudan Black — 66015	SYPRO® Orange Protein Gel Stain — S 5692	SYPRO® Red Protein Gel Stain — S 5077	SYPRO® Ruby Protein Blot Stain — S 4817	SYPRO® Ruby Protein Gel Stain — S 4942	SYPRO® Tangerine Protein Gel Stain — S 5942
Method	Colorimetric	✓	✓	✓	✓	✓	✓	★	✓			✓		✓	✓	✓	✓	✓	✓	✓					
	Fluorescent										✓	✓									★	★	★	★	★
Detection Targets	Proteins		✓	✓	✓	✓	✓	★	✓		✓			✓		✓	★	★	✓		✓	✓	✓	✓	✓
	Glycoproteins	✓										★	★											✓	
	Lipids and/or Lipoproteins														★					✓				✓	
	Nucleic Acids																	✓	✓						
	Phospholipids, Neutral Fats, & Sterols																			★					
Compatibility	PAGE	✓	✓	✓	✓		✓	✓	✓	✓		✓	✓				✓	✓	✓	✓	✓	✓		✓	✓
	IEF (Acrylamide)			✓	✓	★			✓															✓	
	Agarose Gel		✓		★															✓					
	PVDF Membrane			✓	★			✓				✓	✓			✓			✓				✓		
	Cellulose Acetate Membrane														★	✓				✓					
	Nitrocellulose Membrane	✓	✓											✓		★			✓				✓		
	Nylon Membrane																		★						
	MALDI-MS			✓	✓			✓				✓					✓	★					✓	✓	✓
Special Features	High Sensitivity (10 ng)							★																	✓
	Higher Sensitivity (1 ng)																				✓	✓	✓		
	Highest Sensitivity (0.1 ng/mm²)																★	★							
	Fixing Reagent			✓	✓			✓		★							✓	✓			✓				✓
	Requires No Destaining							★									✓	✓			✓				✓
	Rapid Development (5 minutes)		✓													✓									
	Reversible						✓									✓		✓	★						

Protein Analysts

SIGMA

Stain	Sensitivity
Coomassie Brilliant Blue	~30-50 ng
Sypro Ruby Fluorescent	~1-10 ng
Silver Stain	~0.5 – 1.5 ng

TINCION DE GELES CON PLATA

1.- FIJAR 1 HORA O TODA LA NOCHE CON METANOL AL 50% - ACIDO ACETICO AL 10%

2.- FIJAR DURANTE 30 MIN EN ETANOL AL 10% MAS ACIDO ACETICO AL 5%.

3.- LAVAR CON AGUA DESTILADA DURANTE 30 MINUTOS.

4.- DTT (3mg/200ml de agua destilada) DURANTE 30 MINUTOS.

5.- ENJUAGAR SUAVEMENTE EN AGUA DESTILADA.

6.- TEÑIR CON NITRATO DE PLATA AL 0.2% (A G U A D E S I O N I Z A D A) POR 30 MIN.

7.- LAVAR CON AGUA DESTILADA SUAVEMENTE.

8.- REVELAR CON CARBONATO DE SODIO AL 3% MAS FORMALDEHIDO 1 ml /2 LITROS (utilizar un poco y sustituir por fresco) DE 10 A 15 MINUTOS DE REVELADO.

9-- DETENER EL REVELADO CON ACIDO ACETICO AL 1%

10.-LAVAR CON AGUA DURANTE 10 MINUTOS.

11.- GLICEROL AL 3% DURANTE 30'

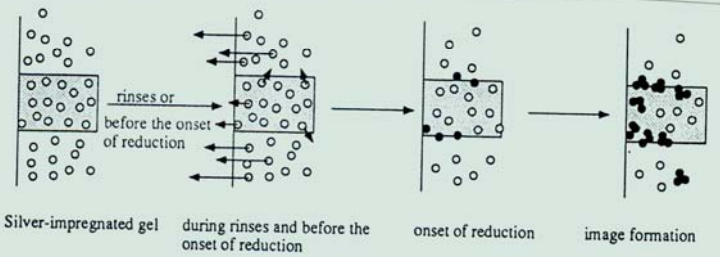
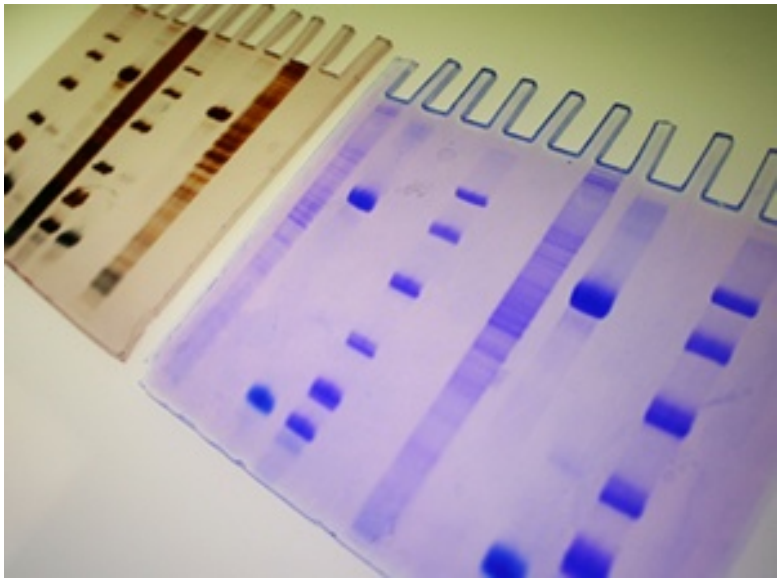


Fig. 2 *Schematic representation of a positive silver-stain.* Symbols as in fig. 1. The long arrows show the important silver ion diffusion out of the gel, while the short arrows show the weak diffusion from the proteins due to silver binding.



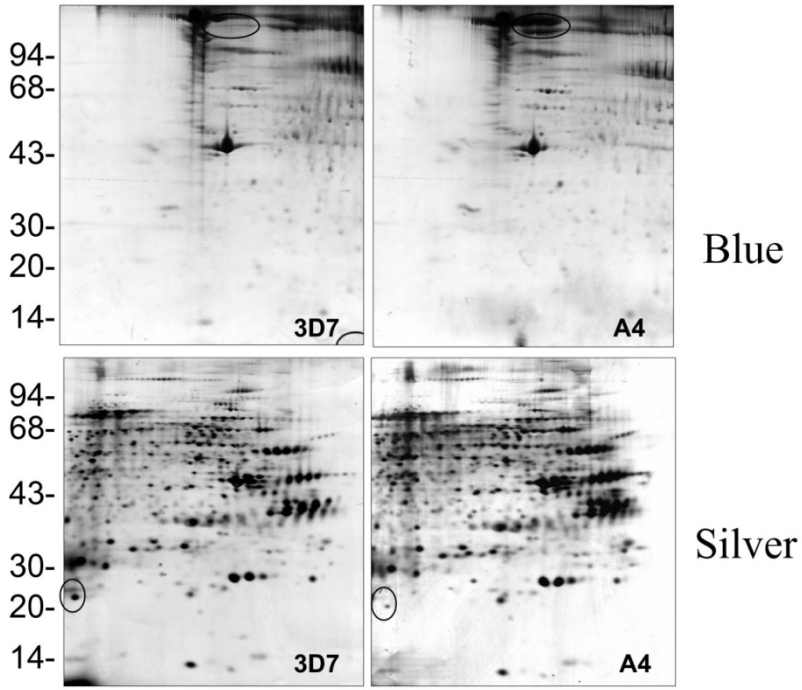


Table 7.1. Radioisotopes Commonly Used in Autoradiography[a]

Isotope	Radiation	Energy (MeV)	Half-life
^{3}H	β	Low (0.018)	12.26 years
^{14}C	β	Medium (0.156)	5730 years
^{35}S	β	Medium (0.167)	88 days
^{45}Ca	β	Medium (0.256)	165 days
^{32}P	β	High (1.71)	14.3 days
^{125}I	γ	High (0.035)	60 days
	X	(0.027)	
	e^{-}	(0.30)	

[a]Modified from Hahn (1983) and Freifelder (1976).

Table 7.2. Recommended Conditions for Optimal Sensitivity of Exposure Using Different Radioisotopes for Autoradiography

Radioisotope	Exposure	Temperature	Type x-ray film
^{3}H	Fluorographic	−70°C	High sensitivity to UV and blue spectrum
^{35}S, ^{14}C	Fluorographic	−70°C	High sensitivity to UV and blue spectrum
^{35}S, ^{14}C	Direct exposure	20°C	High speed
^{32}P	Direct exposure	20°C	High speed
^{32}P	With intensifying screen	−70°C	High sensitivity to UV and blue spectrum
^{125}I	Direct exposure	20°C	High speed
^{125}I	With intensifying screen	−70°C	High sensitivity to UV and blue spectrum

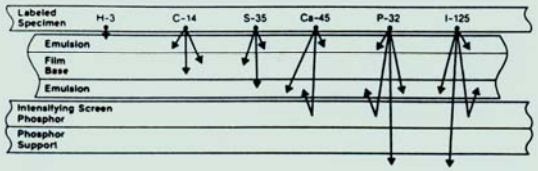
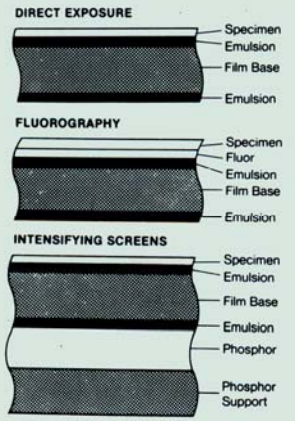
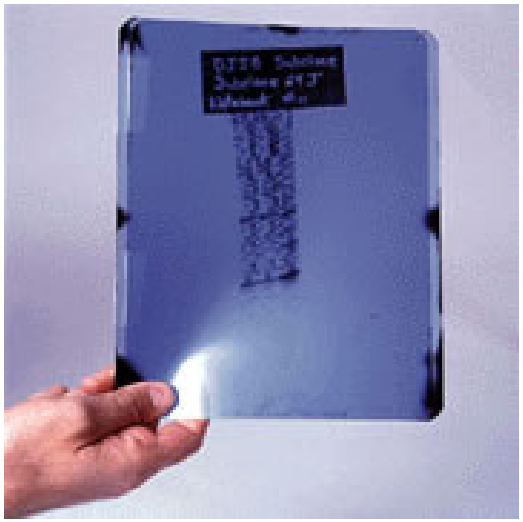


Figure 7.2. Penetration levels of isotopes commonly used for autoradiography. (From Hahn, 1983.)



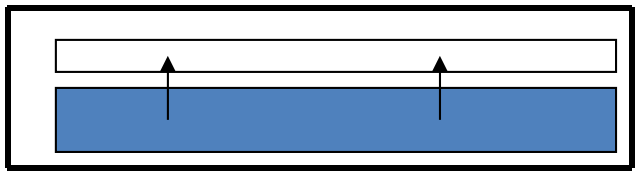


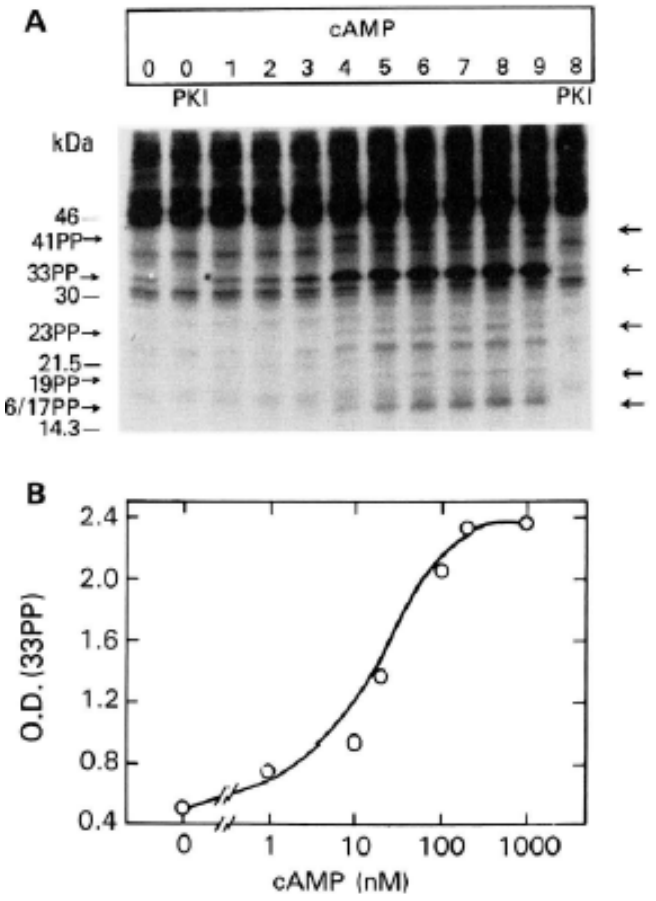
AUTORADIOGRAFIA

SUSTRATOS DE FOSFORILACION ^{32}P
PROTEINAS TOTALES ^{35}S-met

FOSFORO-32

EXPERIMENTO





FILM

REVELADO FOTOGRAFICO

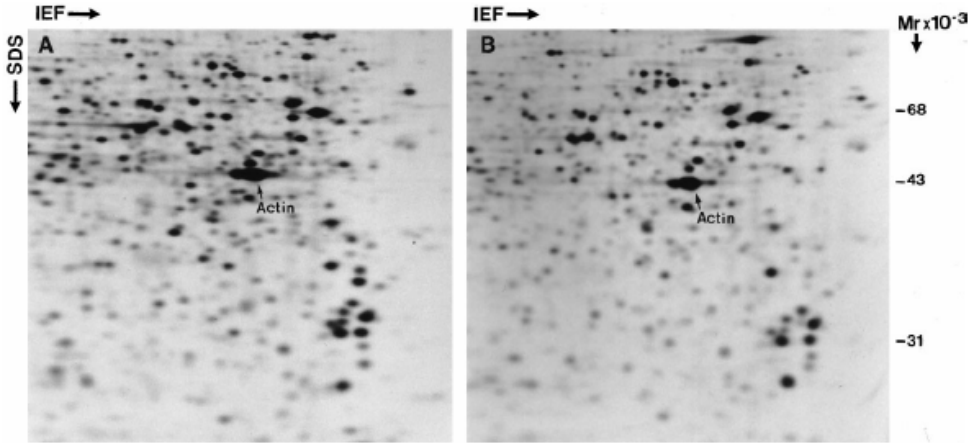
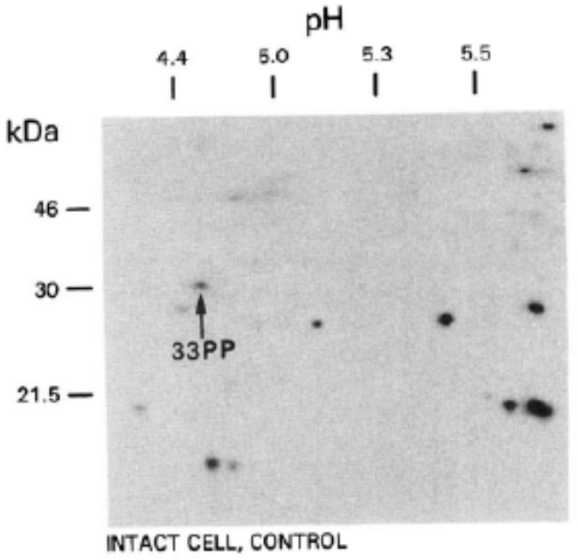


Fig. 9. [^{35}S]Methionine labeled proteins from newborn mouse kidney (A) and (B) lung. Only a fraction of the IEF gels are shown.

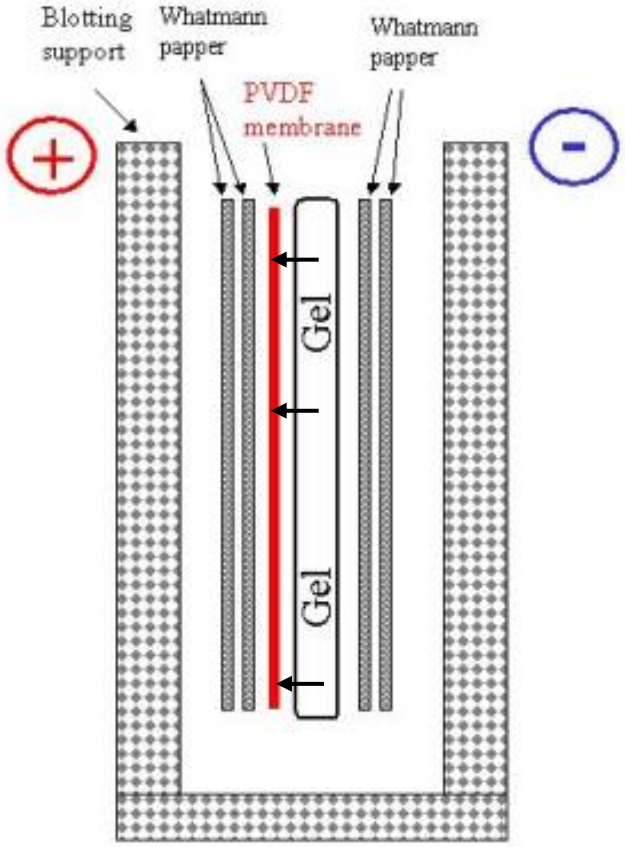
MARCAJE CON 35S-MET

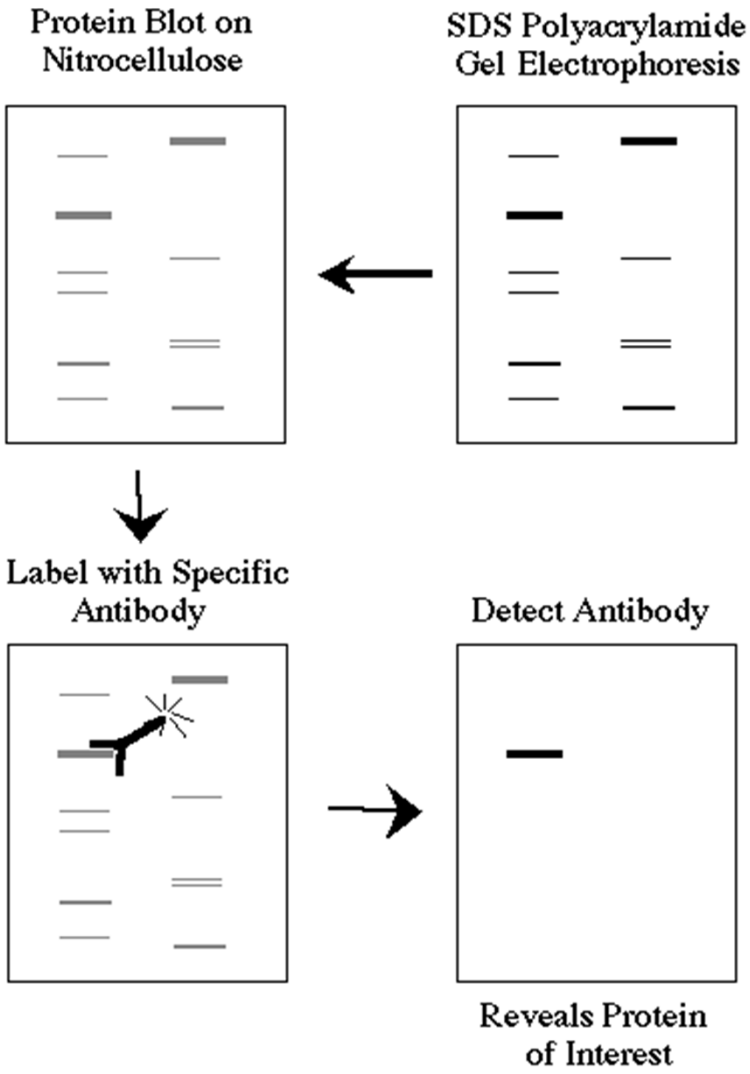


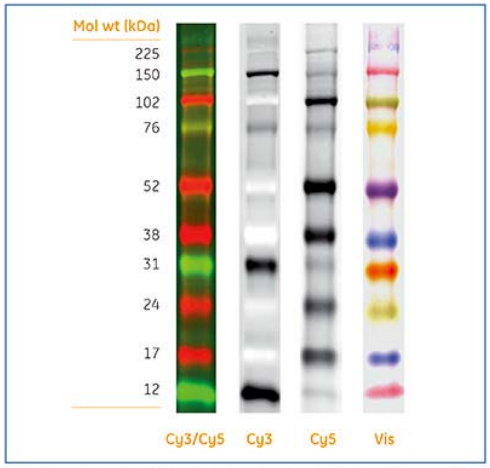
MARCAJE CON $^{32}PO_4$

ELECTROTRANSFERENCIA DE PROTEINAS DESDE GELES DE POLIACRILAMIDA A MEMBRANAS DE NITROCELULOSA

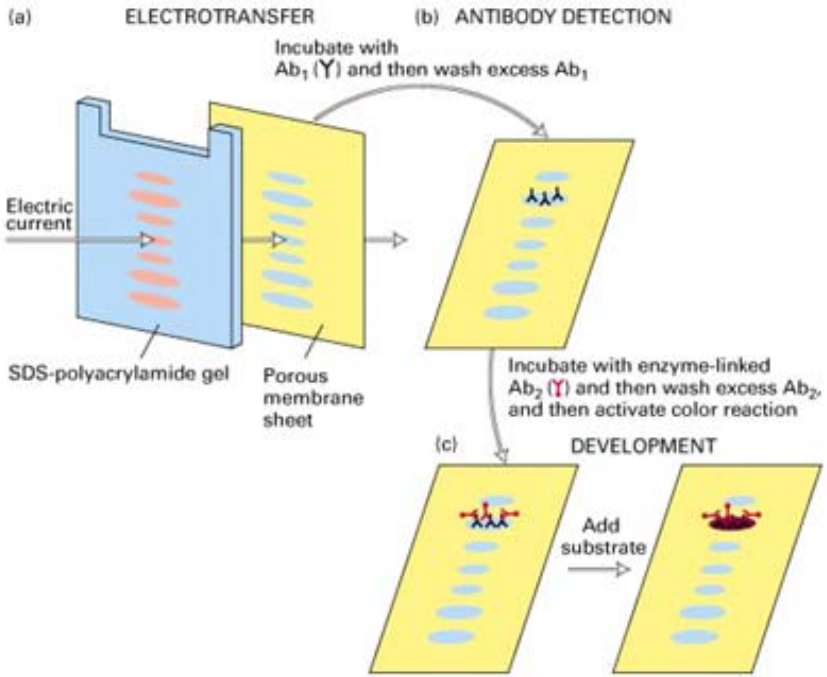
WESTERN BLOT

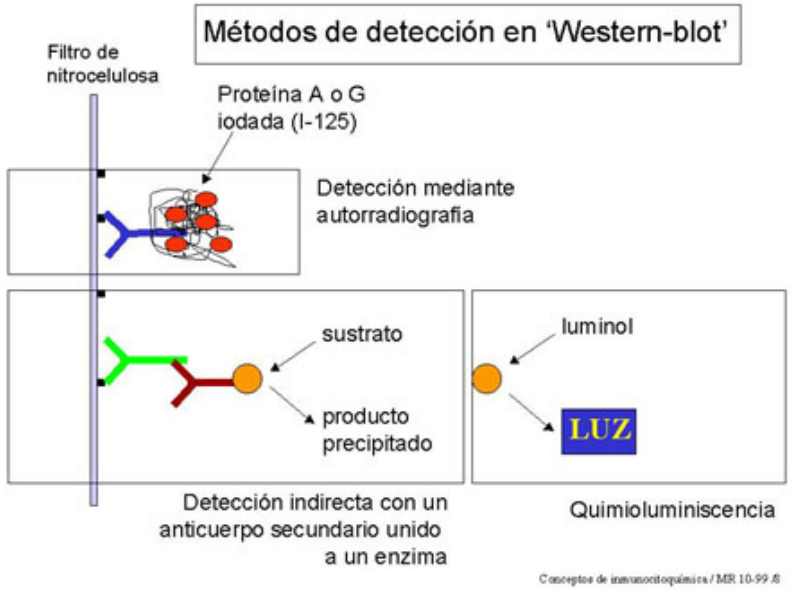


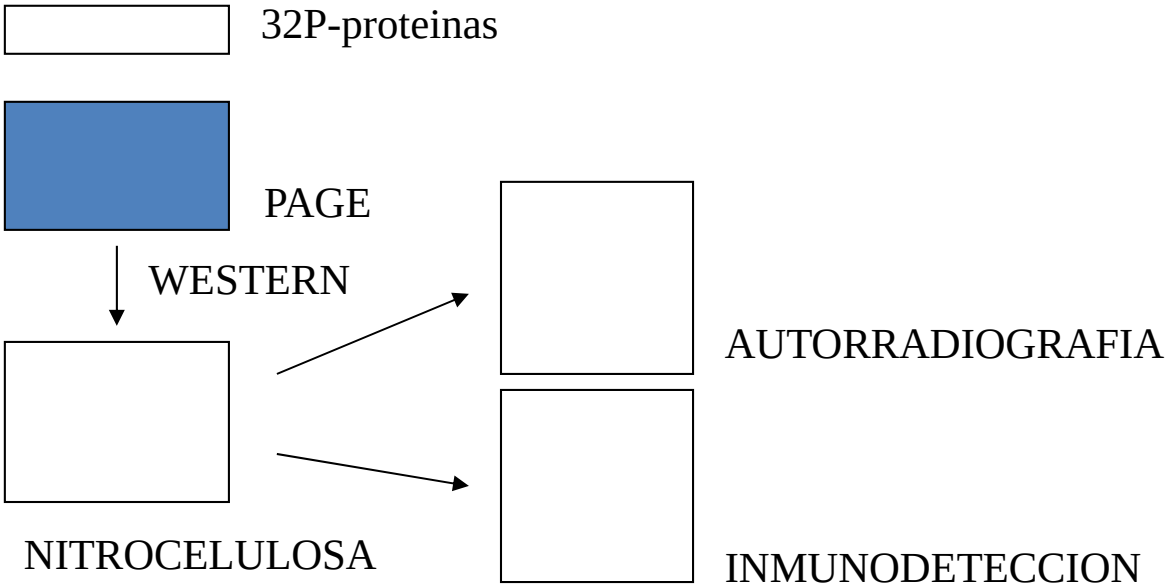


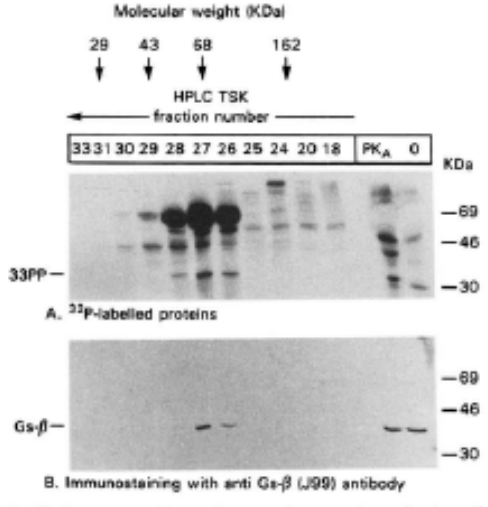


Typhoonで撮影したECL Plex Fluorescent Rainbow Markersです。左から順にFull-color Cy3/G5, Cy3 channel, Cy5 channel, 可視スペクトルになります。

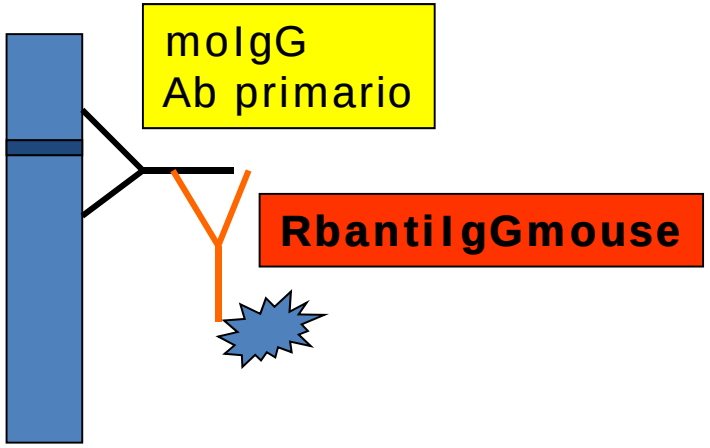

(a) ELECTROTRANSFER
(b) ANTIBODY DETECTION
Incubate with Ab1 (Y) and then wash excess Ab1
Electric current
SDS-polyacrylamide gel
Porous membrane sheet
Incubate with enzyme-linked Ab2 (Y) and then wash excess Ab2, and then activate color reaction
(c) DEVELOPMENT
Add substrate


Métodos de detección en 'Western-blot'
Filtro de nitrocelulosa
Proteína A o G iodada (I-125)
Detección mediante autorradiografía
sustrato
producto precipitado
luminol
LUZ
Detección indirecta con un anticuerpo secundario unido a un enzima
Quimioluminiscencia

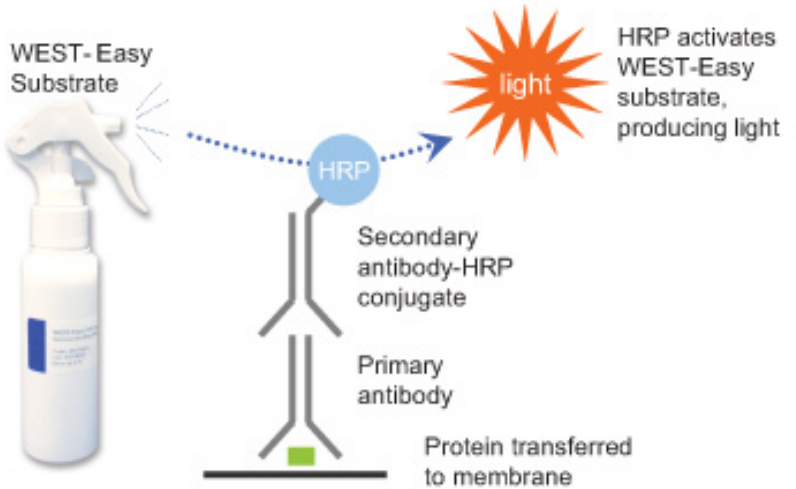

32P-proteinas
PAGE
WESTERN
AUTORRADIOGRAFIA
NITROCELULOSA
INMUNODETECCION

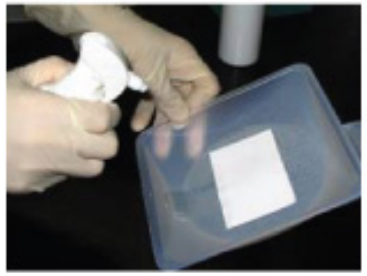

Molecular weight (KDa)
29
43
68
162
HPLC TSK
fraction number
33 31 30 29 28 27 26 25 24 20 18
PKA 0
KDa
69
46
30
33PP
A. 32P-labelled proteins
Gs-β
B. Immunostaining with anti Gs-β (J99) antibody

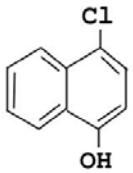
Fluorescencia
Quimioluminiscencia
Actividad enzimática coloreada
(peroxidasa, fosfatasa alcalina)



BLOT





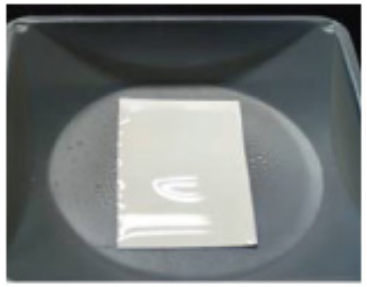


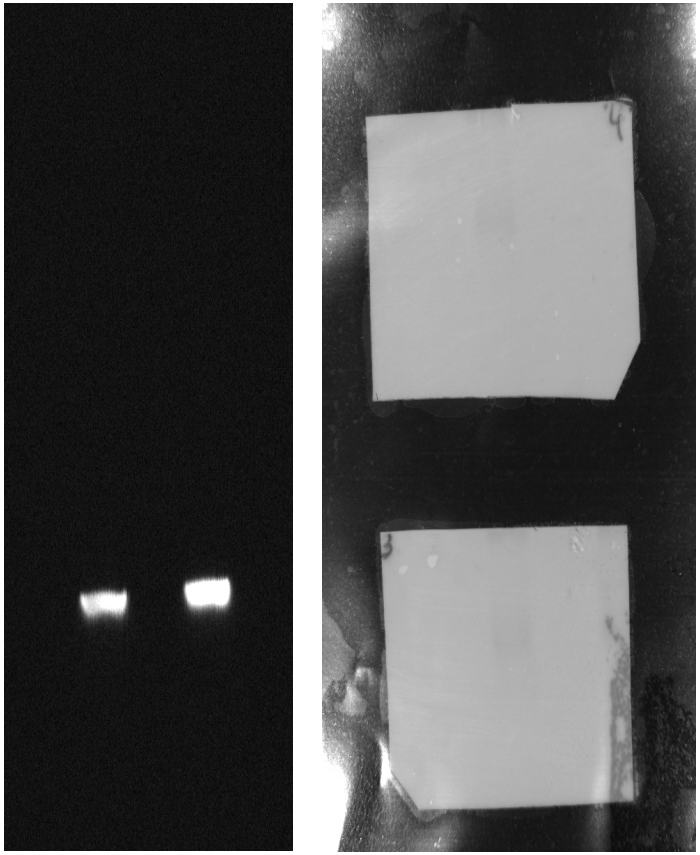
4-chloro-1-naphtol

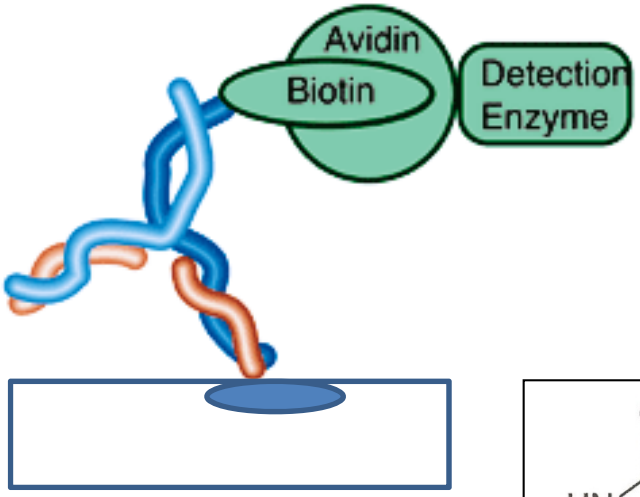
H2O2 HRP

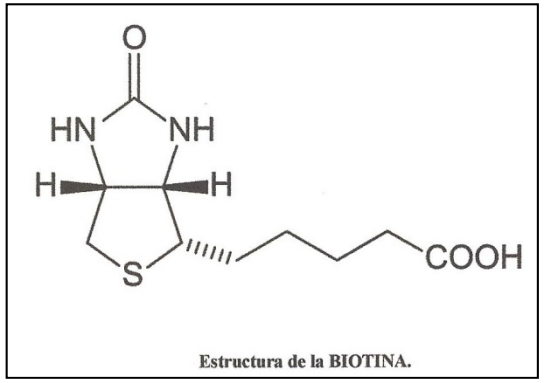
COLOR

$2H_2O_2$ + Luminol → 3'-aminophthalate + N_2 + Light (425 – 510 nm)
HRP (Horseradish Peroxidase)

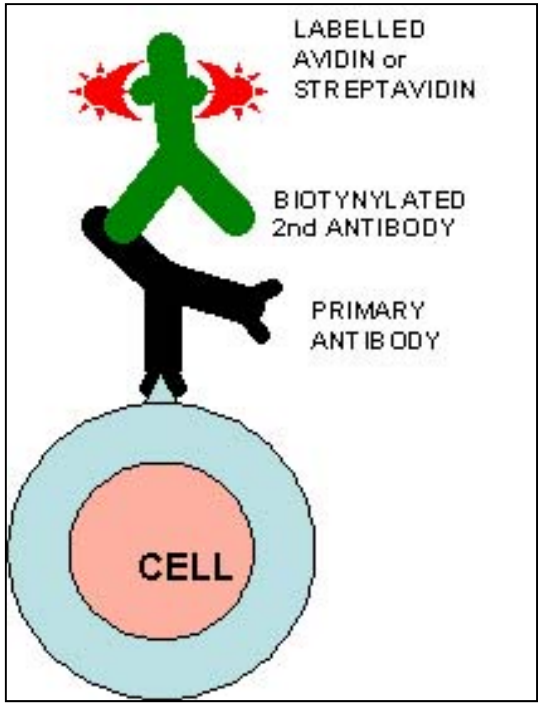








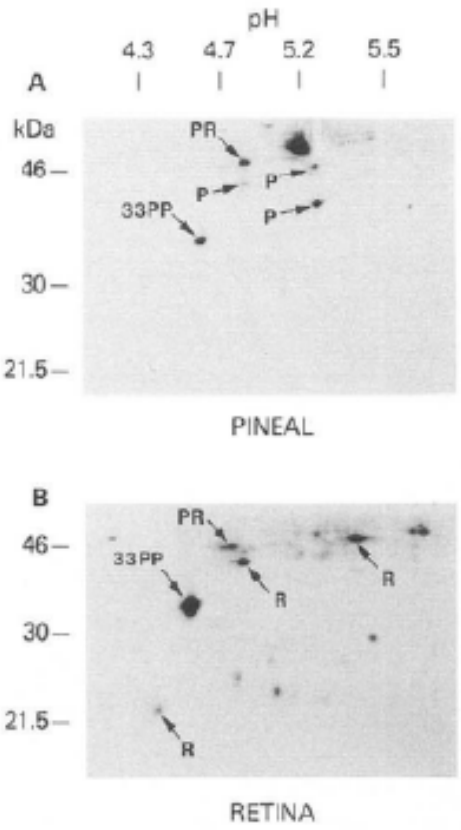
Estructura de la BIOTINA.



AVIDINA
Abunda en la clara de huevo

$Kd = 10^{-15} M$

AVIDINA + BIOTINA ➔ AVIDINA-BIOTINA



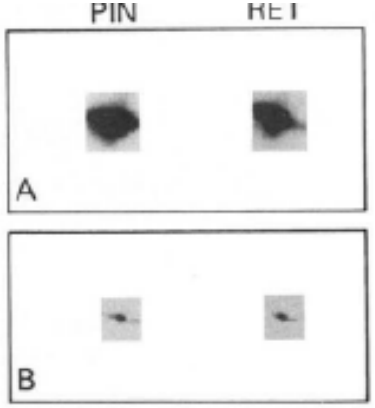


FIG. 11. **Immunological identification of pineal and retinal 33PP in two-dimensional blots as MEKA.** *A*, cytosolic fractions (100,000 × *g*, 60 min) from rat pineal and retina were labeled for 10 min with [γ-^{32}P]ATP in the presence of the catalytic subunit of the PKA and analyzed by two-dimensional-PAGE electroblotting and autoradiography. *B*, sections of the blots in *A* containing 33PP were stained immunologically using an anti-MEKA antiserum as described in the legend to Fig. 10. These results were confirmed in a second study.

Acknowledgments—We want to express our appreciation to the following for their assistance in obtaining antisera used in this report: K. Catt (National Institute of Child Health and Human Development), H. C. Hemmings and P. Greengard (The Rockefeller University), Allen Speigel (National Institute of Diabetes and Digestive and Kidney Diseases), Blake Kapinsky (Biogen), Toshimi Shinohara (National Eye Institute), and Tohru Abe (Akikta University School of Medicine). In addition, we would like to express our appreciation to A. Speigel and T. Shinohara for their critical and creative input during this study and to P. Roseboom (National Institute of Child Health and Human Development) for the sequence analysis.

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- Targets D - F
- Targets G - I
- Targets J - L
- Targets M - O
- Targets P - R
- Targets S - U
- Targets V - Z

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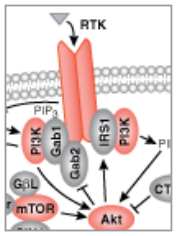
- Growth Factors & Cytokines
- Inhibitors / Stimulators



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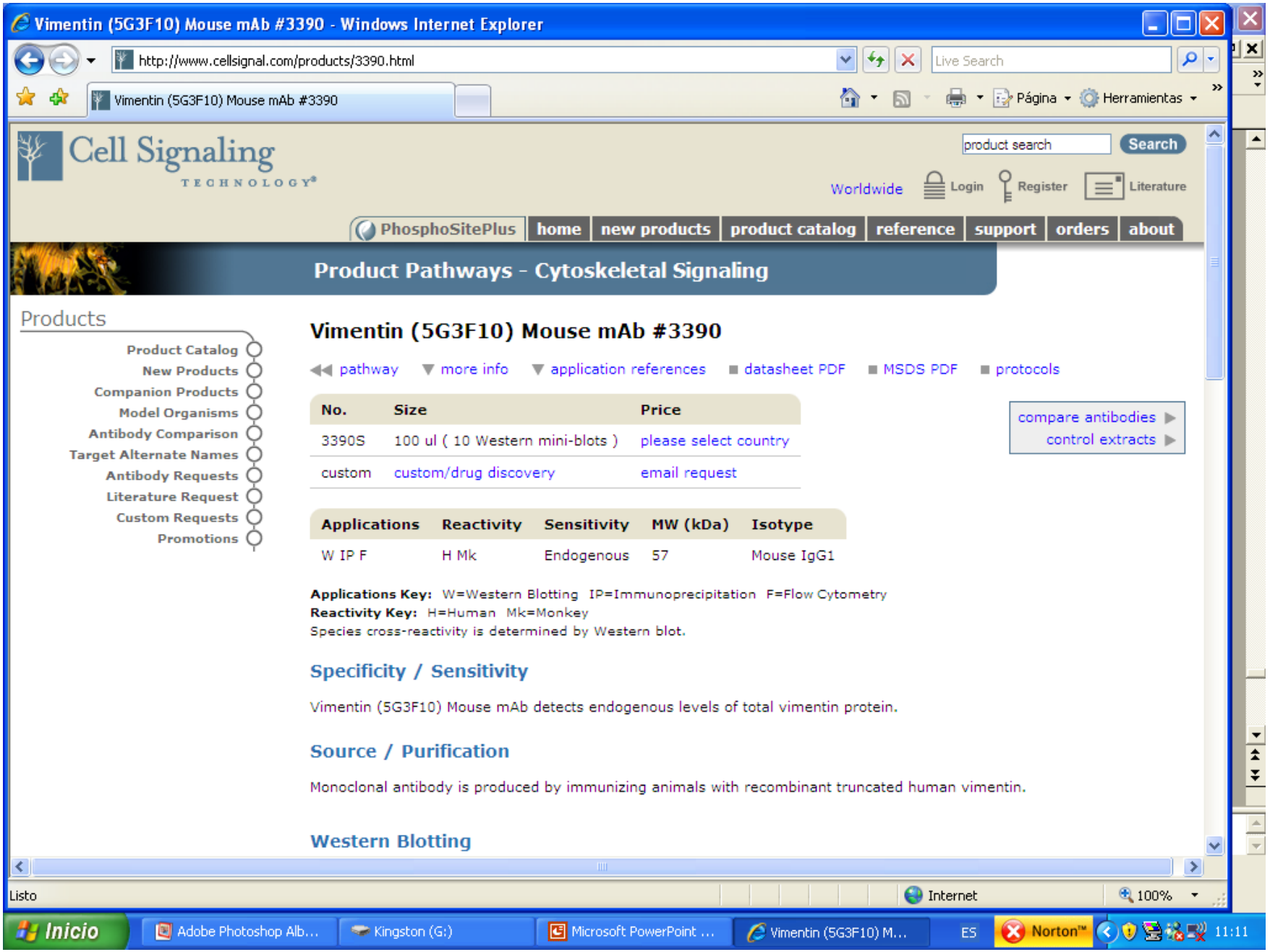
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application references
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custom
custom/drug discovery
email request
compare antibodies
control extracts
Applications
Reactivity
Sensitivity
MW (kDa)
Isotype
W IP F
H Mk
Endogenous
57
Mouse IgG1
Applications Key: W=Western Blotting IP=Immunoprecipitation F=Flow Cytometry
Reactivity Key: H=Human Mk=Monkey
Species cross-reactivity is determined by Western blot.
Specificity / Sensitivity
Vimentin (5G3F10) Mouse mAb detects endogenous levels of total vimentin protein.
Source / Purification
Monoclonal antibody is produced by immunizing animals with recombinant truncated human vimentin.
Western Blotting
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- Affinity Purified anti-Mouse ? (kappa chain specific) [Rabbit]

Gene Name: N/A	**Gene Name Synonym:** N/A	
Gi #: N/A	**NCBI Acc #:** N/A	**Swiss Prot Acc #:** N/A
Length (aa): N/A	**Mol. Weight (Da):** N/A	**Chrom Location:** N/A

- **Antigen:** Kappa Light Chain
- **Immunogen:** Mouse ? (kappa) light chain fragment
- **Family:** Affinity Purified
- **Fraction:** Affinity Purified IgG
- **Purity Note:** This product was prepared from monospecific antiserum by immunoaffinity chromatography using antigens coupled to agarose beads followed by solid phase adsorption(s) to remove any unwanted reactivities. Assay by immunoelectrophoresis resulted in a single precipitin arc against anti-Rabbit Serum, Mouse IgG and Mouse Serum.Specificity was confirmed by ELISA at less than 1% cross reactivity against other mouse heavy or light chain isotypes.
- **Application:** Suitable for immunoprecipitation, immunodiffusion, conjugation and most immunological methods requiring lot-to-lot consistency, high titer and specificity.
- **Recommended Dilution:** ELISA 1:600,000
  WESTERN BLOT 1:5,000 - 1:50,000
  IMMUNOHISTOCHEMISTRY 1:1,000 - 1:5,000
  OTHER APPLICATIONS User Optimized
- **Physical State:** Liquid (sterile filtered)

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  - Arrays & Interaction Profiling
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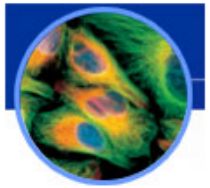
- Core Bioreagents
- Custom Oligos

Antibodies

Secondary Antibodies



Secondary Antibodies.

Antibody Explore Home

- By Animal
- By Label
- Protein A, G and L Detection Reagents
- See also: Protein A, G and L Resins

Secondary antibodies are other host antibodies that bind to primary antibodies or antibody fragments. They are typically labeled with probe that make them useful for detection, purification or sorting applications. Secondary antibodies may be polyclonal antibodies or monoclona antibodies. Polyclonal antibodies lack the specificity of monoclonal antibodies but frequently have higher sensitivities because polyclonal antibodies are a mixture of antibodies that may include very high affinity antibodies. Cloning may not effectively select the highest affinity antibody from a polyclonal host. Secondary antibodies are available with specificity for whole Ig molecules or antibody fragments such as the Fc or Fab regions.

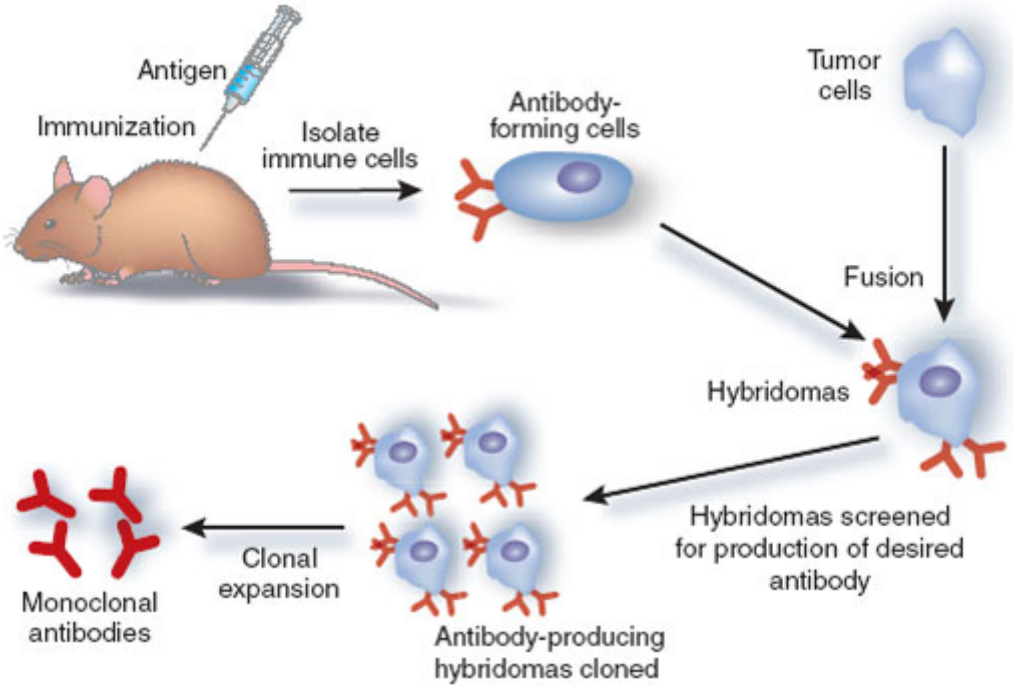
Sigma's polyclonal secondary antibodies are produced from the serum of host animals including mouse, rabbit, goat and sheep. Monoclonal secondary antibodies are produced from mouse hybridoma clones. Secondary antibodies are used in immunodetection, and immunoaffinity purification applications. Immunodetection applications include enzyme linked immunosorbent assays (ELISA); Western blotting; immunohistology, immunoblotting, immunostaining and cell based assays, such as cell-based immunochemical assays and high throughput cell-based screening assays (HTS). Secondary antibodies are useful for cell sorting, fluorescence activated cell sorting, FACS.

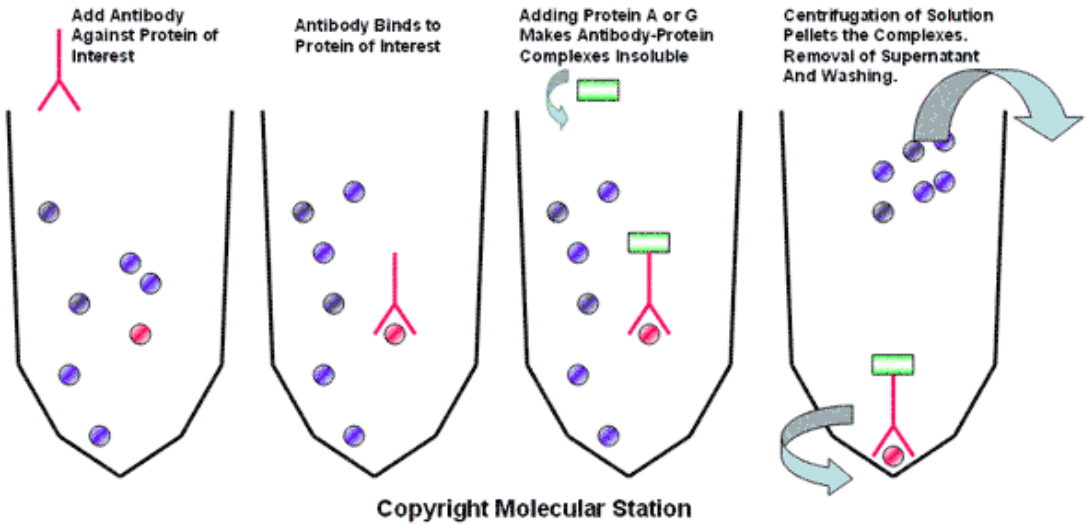
The specific utility of a secondary antibody depends upon its conjugated probe(s). Probes are molecules that support various detection

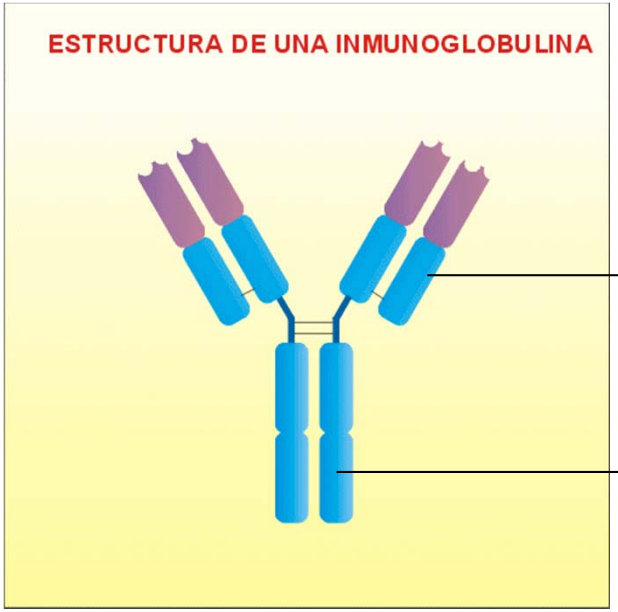
Internet | 100%

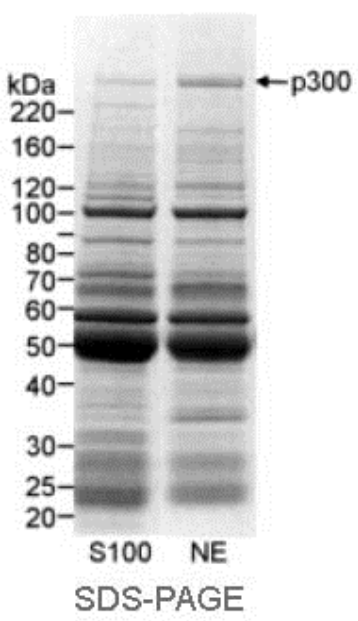
Inicio | Adobe Photoshop Alb... | Kingston (G:) | Microsoft PowerPoint ... | Secondary Antibodies... | ES | Norton™ | 11:26

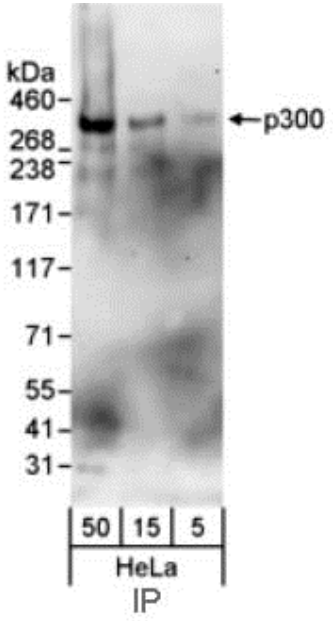
OBTENCION DE ANTICUERPOS



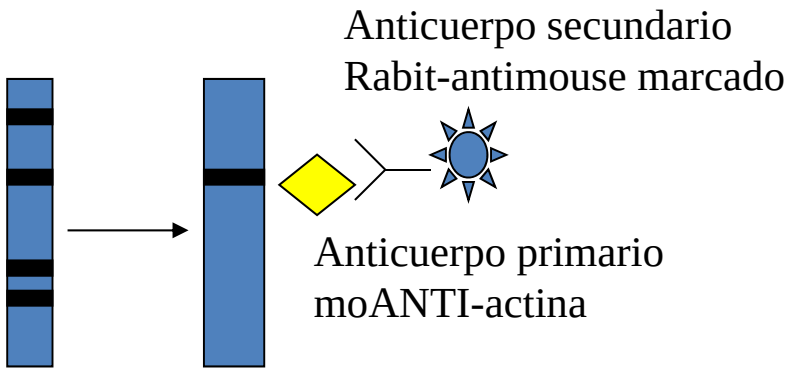

Add Antibody Against Protein of Interest
Antibody Binds to Protein of Interest
Adding Protein A or G Makes Antibody-Protein Complexes Insoluble
Centrifugation of Solution Pellets the Complexes. Removal of Supernatant And Washing.
Copyright Molecular Station


ESTRUCTURA DE UNA INMUNOGLOBULINA
23kDa
53-75kDa


kDa
220
160
120
100
80
70
60
50
40
30
25
20
p300
S100
NE
SDS-PAGE

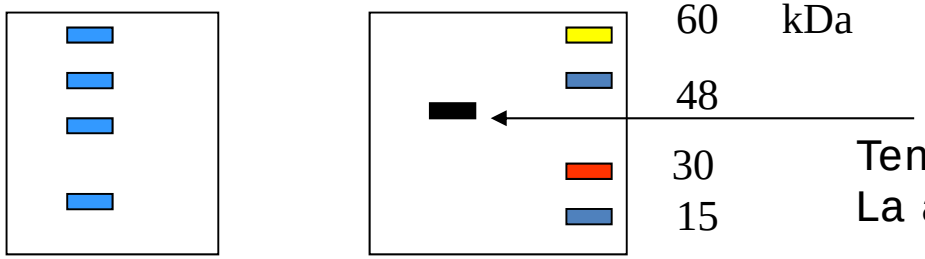

kDa
460
268
238
171
117
71
55
41
31
p300
50
15
5
HeLa
IP

¿En que se basa el marcaje inmunológico de una proteína tras la separación electroforética, por la técnica denominada “western blot”?
¿Que pasos y materiales se requieren para detectar (o descartar) la presencia de una proteína concreta, por ejemplo ACTINA, en una solución tras electroforesis en SDS-poliacrilamida?.

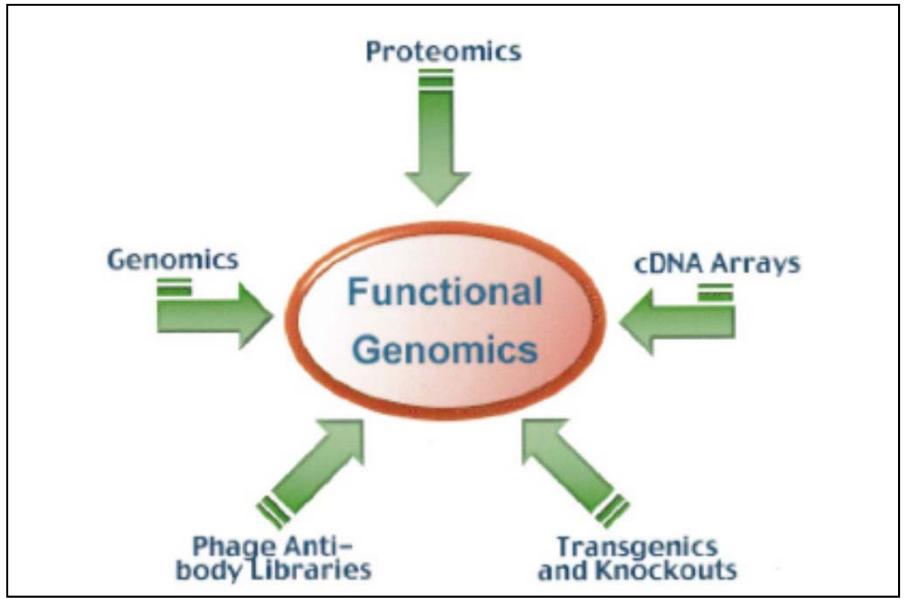


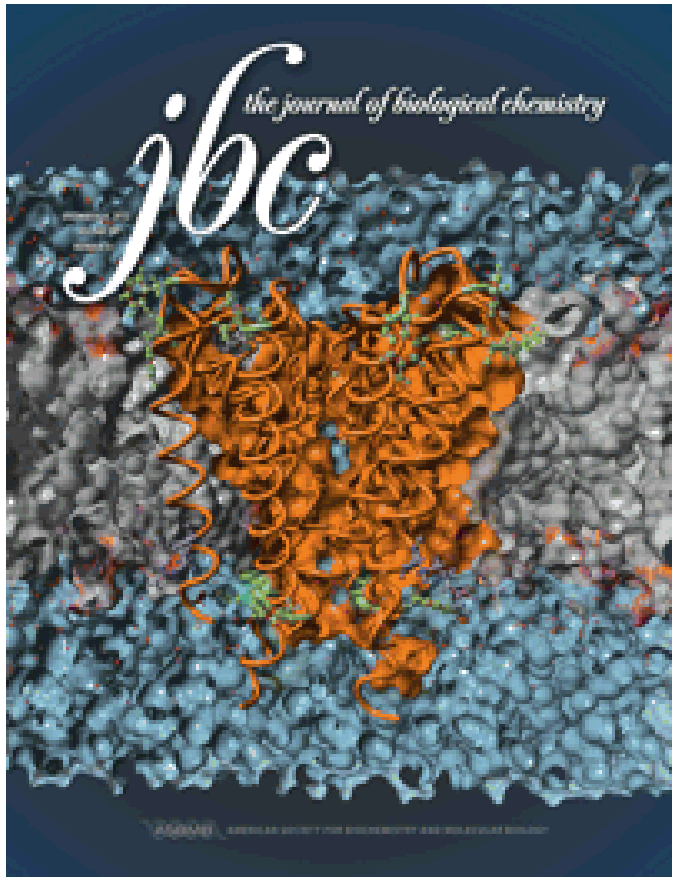
Electrotransferencia➔ membrana de nitrocelulosa

Rainbow markers



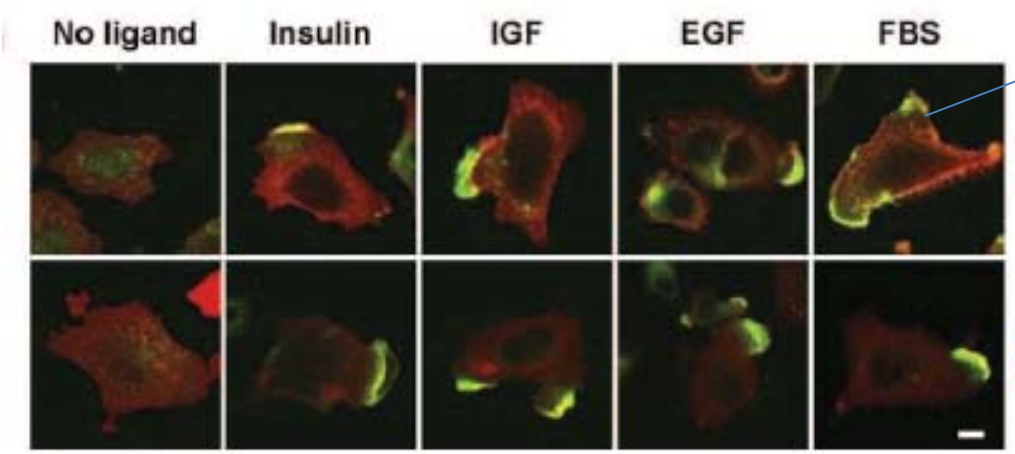
Tener una banda de un pm semejante a
La actina no identifica a la proteína


Proteomics
Genomics
Functional Genomics
cDNA Arrays
Phage Anti-body Libraries
Transgenics and Knockouts

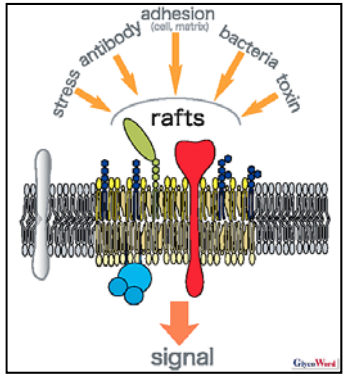

the journal of biological chemistry
jbc

Cell-surface Receptor for Complement Component C1q (gC1qR) Is a Key Regulator for Lamellipodia Formation and Cancer Metastasis

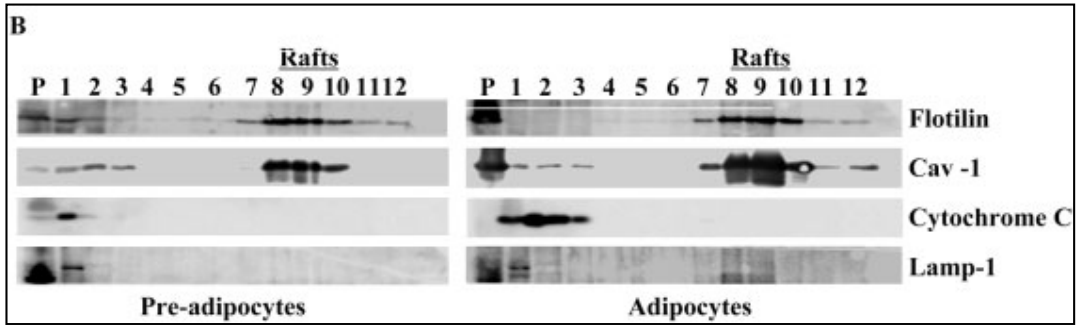
THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 286, NO. 26, pp. 23093–23101, July 1, 2011

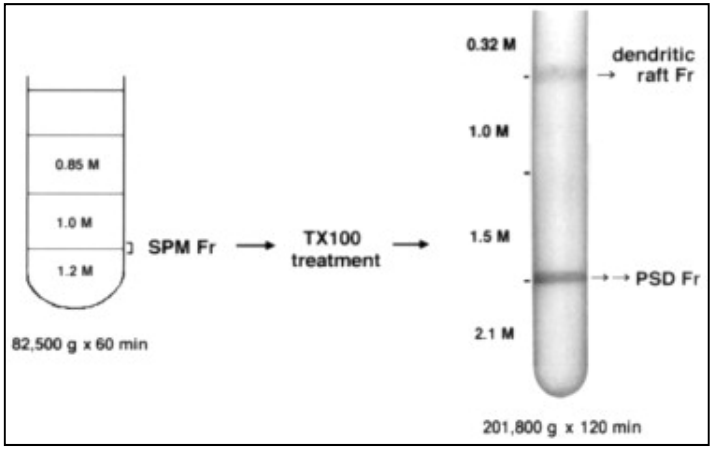


Lamelipodios➔prolongaciones transitorias

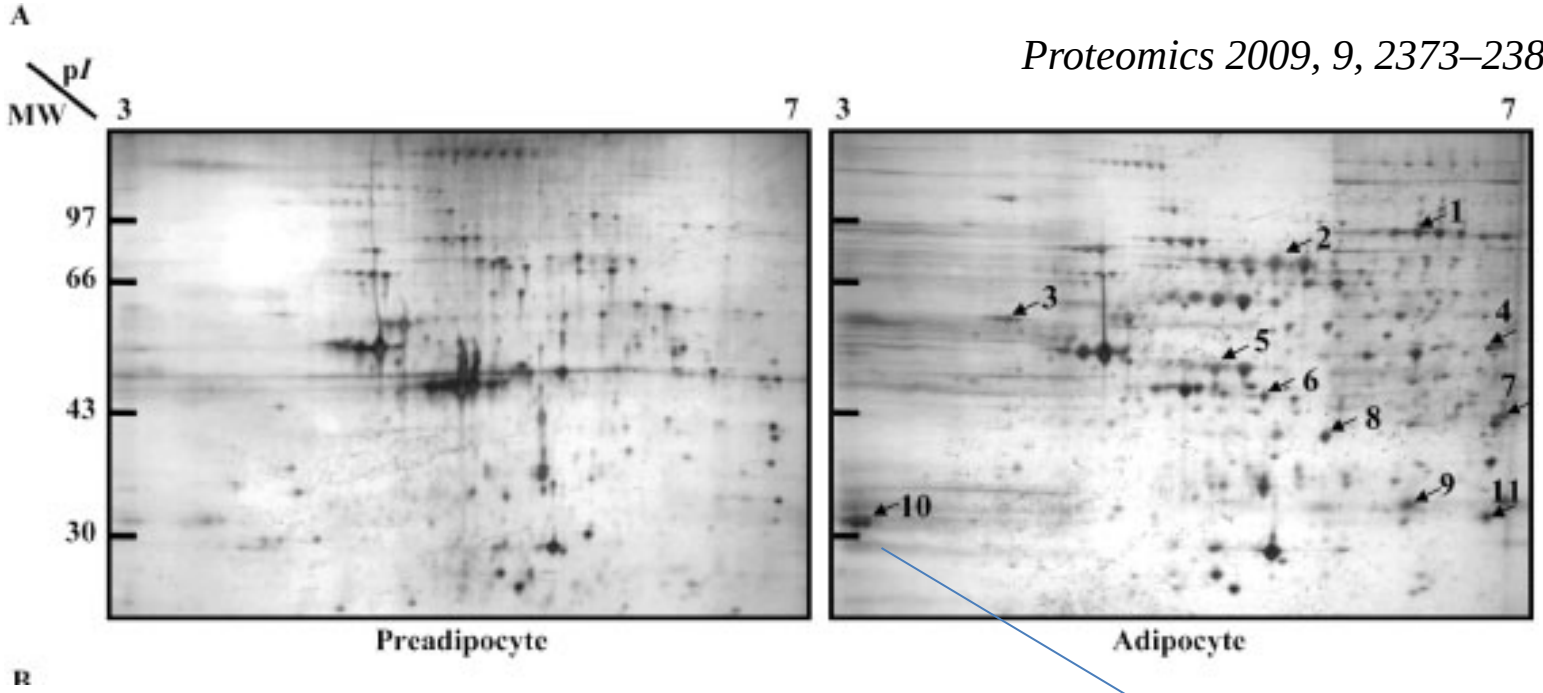


A549 cells de tumor de pulmón





Proteomics 2009, 9, 2373–2382



B

A

```
  1 SARGHTVPWP GRPSLCTCPP RDAPSAALRA PRPRRRRPPA SRTAIPAQPL
 51 RHLLQPAPRP CLRPFGLLSV RAGSARRSGL LQPPVPCACG CGALHTEGDK
101 AFVEFLTDEI KEEKKIQKHK SLPKMSGDWE LEVNGTEAKL LRKVAGEKIT
151 VTFNINNSIP PTFDGEEEPS QGQKAEEQEP ELTSTPNFVV EVTKTDGKKT
201 LVLDCHYPED EIGHEDEAES DIFSIKEVSF QATGDSEWRD TNYTLNTDSL
251 DWALYDHLMD FLADRGVDNT FADELVELST ALEHQEYITF LEDLKSFVKN
301 Q
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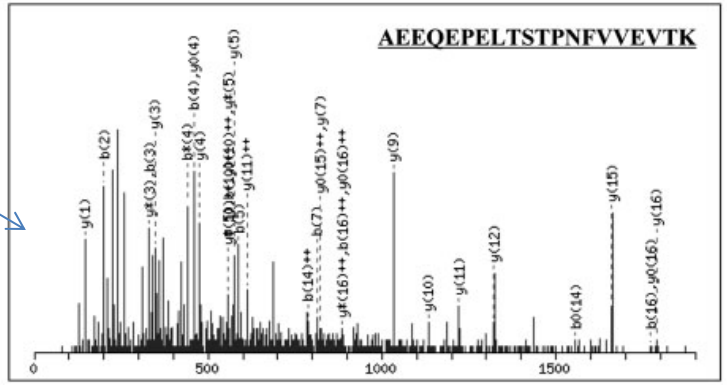
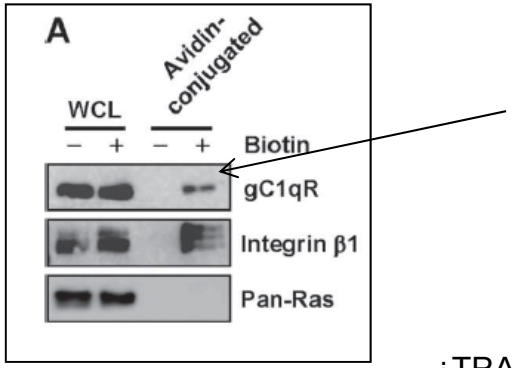
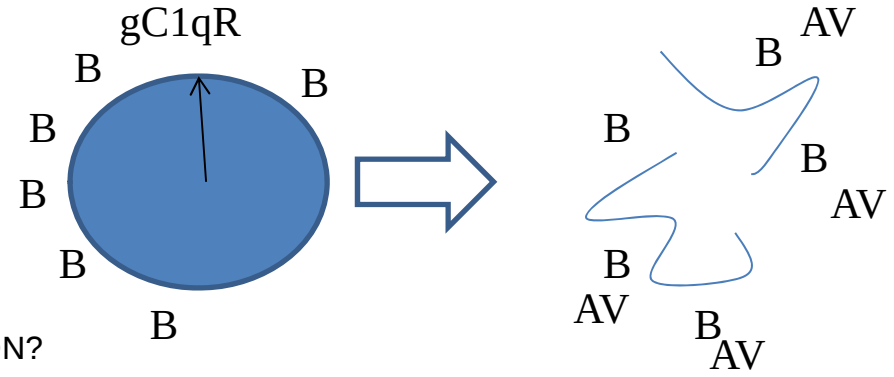
DIGESTIÓN PEPTIDICA
ANALISIS DE PEPTIDOS
POR ESPECTORMETRIA DE MASAS

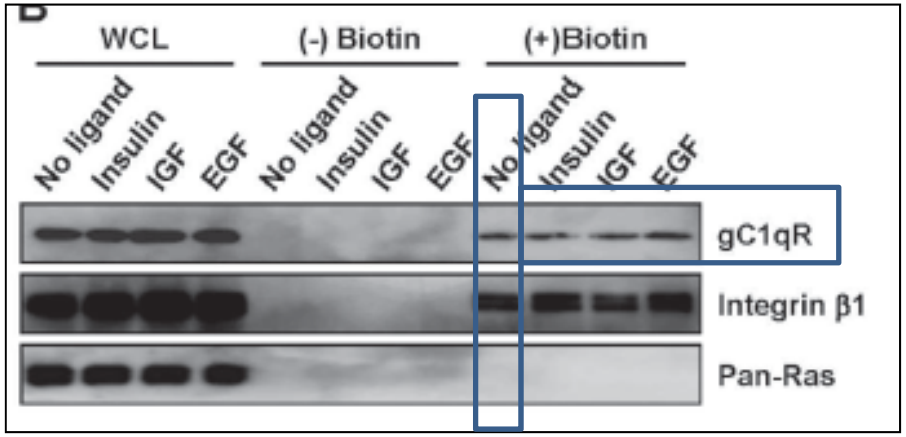


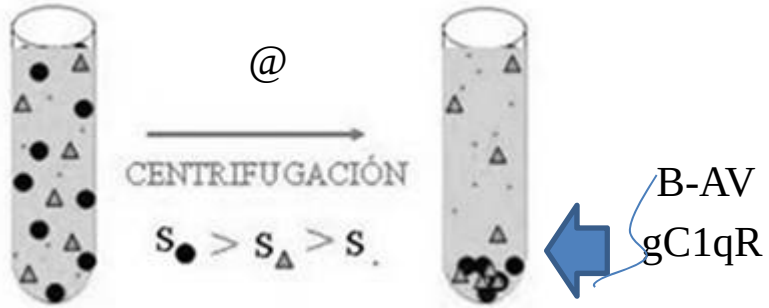
gC1qR*=RECEPTOR PARA EL COMPONENTE DEL COMPLEMENTO C1q*

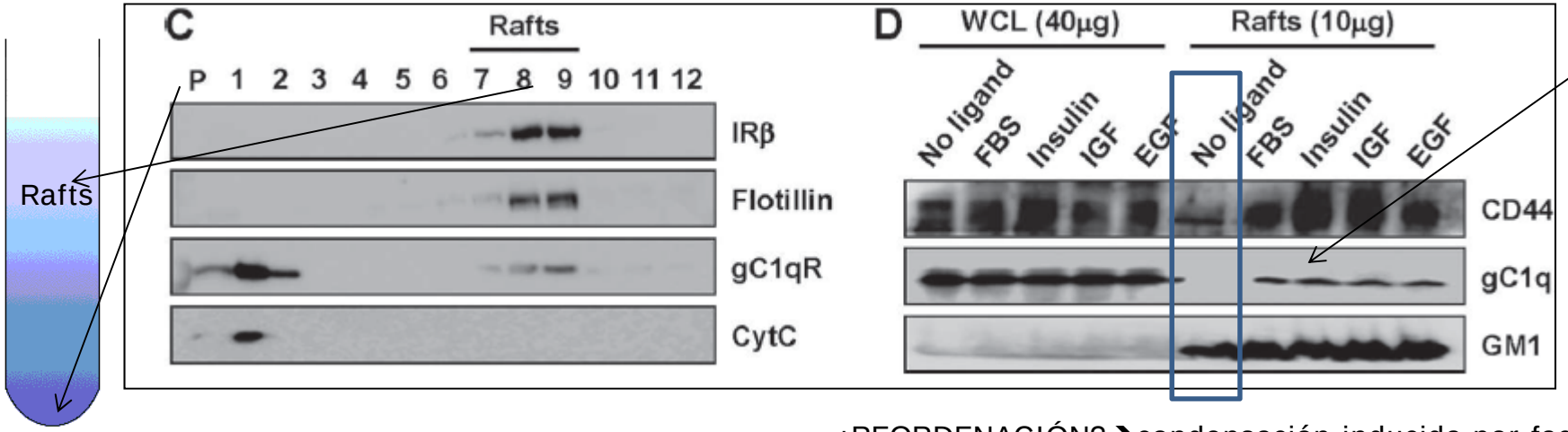




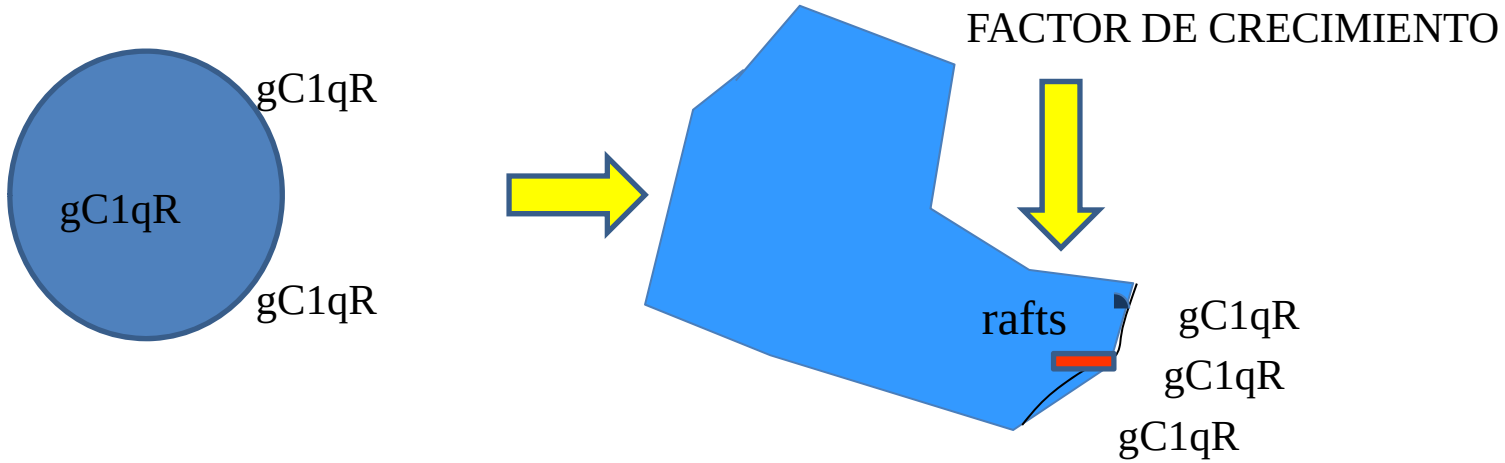
¿TRANSLOCACION?



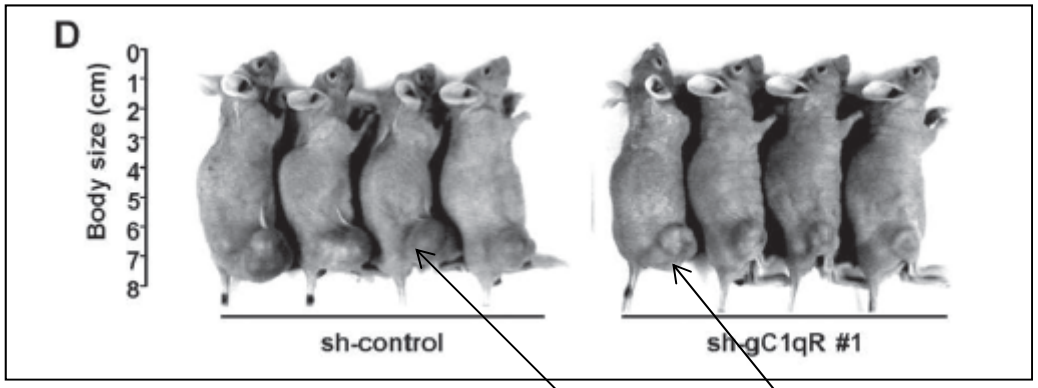


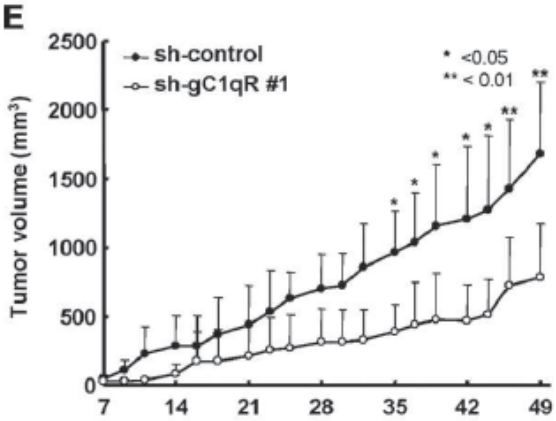


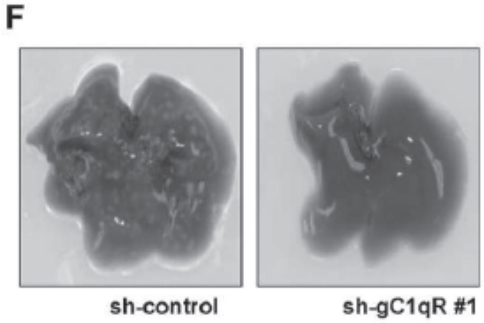
¿REORDENACIÓN?➔condensación inducida por factores

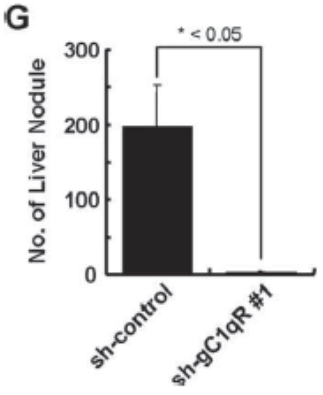


NO EXISTE TRANSLOCACION DE RECEPTORES gC1qR, SINO REORDENACIÓN O AGRUPACIÓN A ZONAS ESPECIFICAS FUNCIONALES D ELA MEMBRANA (RAFTS)



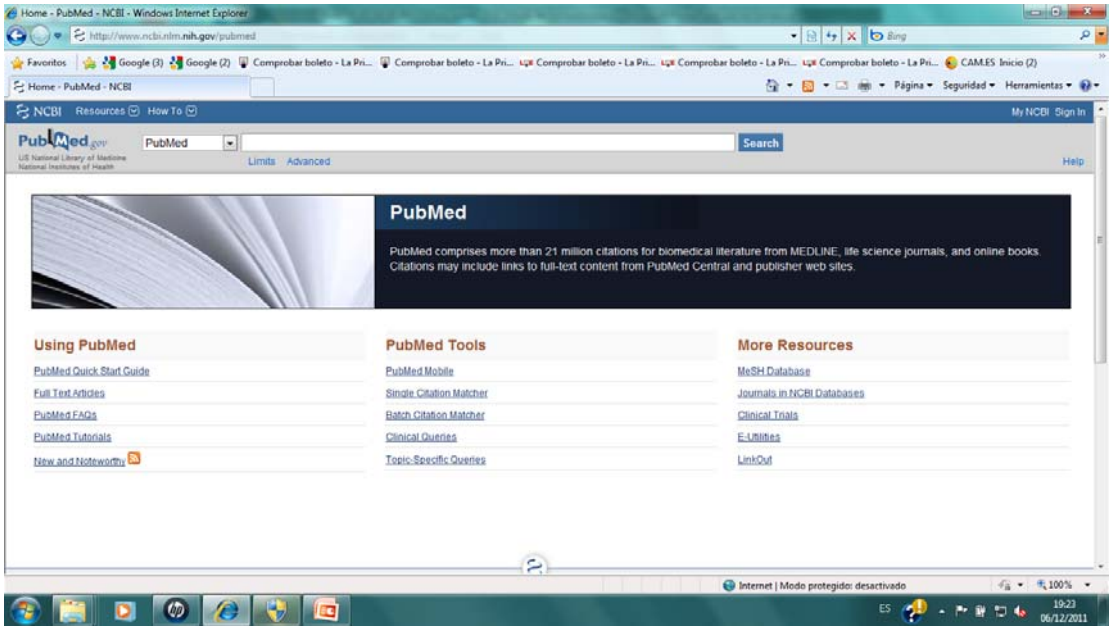






El tañaño del tumor y las metastasis hepáticas Disminuyen con la inyección de las células Knockdown para gC1qR

http://www.ncbi.nlm.nih.gov/pubmed



jareig@umh.es