

Preparacion del medio de cultivo alpha-MEM

Las celulas CHrB30 deben ser cultivadas usando el medio alpha-MEM (Ref. 11900-073, power o 22571-038, liquid).

[LIFE TECHNOLOGIES](#). Para 11900-073, power, seguir este protocolo.

1. Measure out 5% less water(deionised-distilled) than desired total volume of medium, using a mixing container that is a close to final volume as possible.
 2. Add powder medium to water at room temperature (+15 to +20°C) with gentle stirring. (Do not heat water).
 3. Rinse out inside of package to remove all traces of powder.
 4. Add 2.2 g NaHCO₃ per litre of medium.
 5. Dilute to desired volume with water. Stir until dissolved. (Do not over mix).
 6. Ajust pH of medium to 0.3 to 0.3 below desired final working pH*: use of 1N NaOH and 1N ClH is recommended. (Add slowly with stirring). After pH has been adjusted, keep container closed until medium is filtered.
 7. Sterilise immediately by membrane filtration.
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Protocolo de cultivo para CHrB30

- Usar botellas de 75 cm², sin aireacion. El volumen final aproximado es **18 ml**.
 - **14.5 ml** α-MEM [22571-038 [LIFE TECHNOLOGIES](#)] +10% FCS
 - **3.5 ml** penicilina/streptomicina 5000 U/ml
 - 20 µl colchicina 10 mg/ml [Cf = 20 µg/ml]
 - CHrB30 crecen adheridas a la botella. Retirar el medio viejo, lavar con PBS y despegar las celulas con 4 ml de tripsina.
- Añadir las celulas a las nuevas botellas o bien seguir el protocolo para hacer stock.
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