PRIMACYT
Cell Culture Technology

Plating and Culture of Primary Hepatocytes

Updated 29.08.2012
Version 03

Recommended culture conditions

- HPM (Hepatocyte Plating Medium): for attachment of hepatocytes
- HHMM (Human Hepatocyte Maintenance Medium): for serum-free culture of human, monkey, and dog hepatocytes
- HGM (Hepatocyte Growth Medium): for serum-free culture of rat hepatocytes
- Collagen Coated Cell Culture Plates

1. Plating of hepatocytes

- Spin cell suspension (density is approx. 5 x 10^6 cells/ml) at 50 g, 4 °C, 5 min
- Aspirate supernatant
- Add Hepatocyte Plating Medium to get a cell density of 1-2 x 10^6 cells/ml
- Optional: you may add 5 % serum to improve attachment at viabilities below 80 %
- Stain with trypan blue and count the viable and dead cells in a counting chamber
- Calculate cell number and viability
- Adjust cell suspension to the desired density for plating
- Use Collagen Coated Cell Culture Plates
- Recommended cell numbers:

    - 6well: per well 1.2 x 10^6 cells in 1 ml -> set up 1.2 x 10^6 cells/ml
    - 12well: per well 0.6 x 10^6 cells in 1 ml -> set up 0.6 x 10^6 cells/ml
    - 24well: per well 0.3 x 10^6 cells in 0.5 ml -> set up 0.6 x 10^6 cells/ml
    - 96well: per well 0.05 x 10^6 cells in 0.1 ml -> set up 0.5 x 10^6 cells/ml

- Do not reduce the volume of plating medium per well
- Let the cells attach for 3-4 h at 37 °C and 5 % CO_2

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2. Culture of hepatocytes

- After attachment of cells: change medium
- Optional: Washing the cells with warm 0.9 % NaCl or PBS

    - 6well: 2 ml/well
    - 12well: 1 ml/well
    - 24well: 0.5 ml/well
    - 96well: 50 µl/well

- Heat the culture medium to 37 °C (no longer than 15 min)
- Change the Hepatocyte Plating Medium to culture medium (HHMM or HGM)

    - 6well: 1 ml/well
    - 12well: 0.5-1 ml/well
    - 24well: 0.3-0.5 ml/well
    - 96well: 50 µl/well

- Change the medium daily (especially when plated at high cell density)
- Change the medium quickly, do not let the cells dry

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