Thawing, Plating and Culture of Primary Cryopreserved Hepatocytes

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Version 01

Required and recommended media and consumables

- Thawing and Plating Kit consists of
  - HTM: Hepatocyte Thawing Medium
  - HWM: Hepatocyte Washing Medium
  - HPM-cryo: Hepatocyte Plating Medium for cryopreserved hepatocytes
- For the use of cryopreserved hepatocytes in suspension only HTM and HWM are required. Both components are not sold separately, only in combination with the kit.
- MHM (Mouse Hepatocytes Medium): for serum-free culture of mouse hepatocytes (not included in this kit)
- Collagen coated cell culture plates (not included in this kit)

1. Arrival of the cryopreserved cells in your laboratory

- Place the cryogenic vial with frozen hepatocytes immediately into the gas phase of liquid nitrogen tank or store at/below -150 °C

2. Thawing and Plating of primary hepatocytes

- Warm water bath, HTM, and HWM to 37 °C
- Set HPM-cryo to room temperature
- Remove the vial with hepatocytes form liquid nitrogen/-150 °C and place it immediately into the 37 °C warm water bath until the cell suspension is thawed (approx. 1-2 min)
- Spray 70 % ethanol on the cryogenic vial for disinfection
- Transfer the cell suspension into the tube with HTM
- Wash the cryogenic vial with 0.5-1 ml HWM to remove the cells completely and combine it with the cells in the tube
- Add HWM to a final volume of 50 ml
- Rotate the tube slowly two or three times
- Pellet the hepatocytes by centrifugation at 50 x g and 20 °C for 10 min
- Remove the supernatant, gently loosen the cells without any additional medium by gently agitating the bottom of the tube. Do not vortex or shake the cells.
- Wash the loosen cells with 20 ml HWM followed by centrifugation at 50 x g and 20 °C for 5 min
- Remove the supernatant, gently loosen the cells without any additional medium by gently agitating the bottom of the tube. Do not vortex or shake the cells.
- Re-suspend the pellet in an appropriate volume of HPM-cryo; 2 ml of HPM-cryo is recommended when counting one cryogenic vial with hepatocytes, 5-7 mio./vial.

- Determine cell viability and live cell number with the trypan blue exclusion test in a counting chamber (do not use automated cell counter)
- Seed the cells at a density recommended in the corresponding data sheet of each individual lot.
- Adjust cell suspension to the desired density for plating with HPM-cryo

Note: the actual seeding density may vary from lot to lot. The recommended seeding density for each lot is stated on the accompanying data sheet.

- Recommended volume of plating medium per well:
  - 6well: 1-2 ml
  - 12well: 1 ml
  - 24well: 0.5 ml
  - 96well: 100 µl

- Let the cells attach for at least 6-7 h at 37 °C and 5 % CO_2, do not let the cells attach overnight

3. Culture of primary mouse hepatocytes

- After attachment of cells: change medium
- Heat the culture medium to 37 °C (no longer than 15 min)
- Washing step with medium or PBS is not recommended
- Replace the Hepatocyte Plating Medium (HPM-cryo) with Mouse Hepatocytes Medium (MHM)
  - 6well: 1 ml/well
  - 12well: 0.5 ml/well
  - 24well: 0.3 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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