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Cell Culture Technology

Thawing of Primary Cryopreserved Fish Hepatocytes

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Required and recommended media and consumables

- Thawing kit consists of
  - HTM: Hepatocyte Thawing Medium
  - HWM: Hepatocyte Washing Medium
  - L15 Medium (complete) for use of cells in suspension assays

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 of primary fish hepatocytes

- Warm water bath, HTM, and HWM to approx. 15 °C
- Set L15 Medium to approx. 15 °C
- Remove media from water bath and set it to 37 °C
- Remove the vial with hepatocytes from 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 cryo-vial for disinfection
- Transfer the cell suspension into the tube with HTM
- Wash the cryogenic vial 1-2x 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 100 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 L15 medium (see Lot-info for post-thaw yield per vial).
- Determine cell viability and live cell number with the trypan blue exclusion test in a counting chamber
- Adjust cell density according to your experiment

Note: Further studies with fish hepatocytes should be performed within the temperature range of 10-20 °C (optimum is approx. 15 °C).

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