PRIMACYT
Cell Culture Technology

Thawing of Primary Cryopreserved Chicken Hepatocytes

Updated 10.02.2017
Version 02

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.

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 chicken hepatocytes

- Warm water bath, HTM, and HWM to 37 °C
- Set HPM-cryo to room temperature
- 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 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 200 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 100 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 (see Lot-info for post-thaw yield per vial). If cells are intended for use in suspension, HPM-cryo may be replaced by any other suitable medium
- Determine cell viability and live cell number with the trypan blue exclusion test in a counting chamber
- Adjust cell density according to your experiment

FOR IN VITRO RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC PROCEDURES.

PRIMACYT Cell Culture Technology GmbH, Hagenower Str. 73, D-19061 Schwerin, FRG, www.primacyt.com
E-mail: info@primacyt.de, Tel.: +49 (0)385-3993-600, Fax: +49 (0)385-3993-602