

Assay Methods Hepatocyte Protocols

BD Gentest™ CryoHepatocytes Percoll™ Recovery Method

The protocol below describes the procedure for thawing and recovery of cryopreserved hepatocytes using the CryoHepatocytes Percoll Purification kit.

Note: Please follow standard sterility practices for cell culture.

Materials and Equipment:

- BD Gentest™ CryoHepatocyte Purification Kit (BD Biosciences Cat. No. 454500)
- ISOM's Seeding Media: ISOM's media (Tube B) + 10% Fetal Bovine Serum. [Note: If the ISOM's seeding media in BD Gentest™ CryoHepatocyte Purification Kit is not enough, ISOM's media only kit can be ordered separately (BD Biosciences Cat. No.454600). It contains 4 x 50mL ISOM's. If cells are not to be seeded for plating but are to be used in suspension, use Williams E media (Sigma Cat. No. W-1878) for BD-Gentest human CryoHepatocytes or KHB Buffer plus 20 mM HEPES (9.6 gm powder (Sigma Cat. No. K-3753), 4.76 gm HEPES powder, final volume 1000 mL with pH adjusted to pH 7.4) for BD-Gentest animal hepatocytes.].
- 37°C waterbath
- Low speed centrifuge set up at Room Temperature (Settings Example: Model Eppendorf 5810R centrifuge Settings: accelerate 5/break 0)
- Biosafety hood
- BD Falcon™ 2 mL pipet (BD Cat. No. 357507)
- BD Falcon 15 mL Polypropylene centrifuge tube (BD Cat. No. 352097 or 352196)

Procedure

1. Pre-warm Percoll isolation kit Tube A & B, and ISOM's Seeding Media with 10% FBS to 37°C
2. Thaw CryoHepatocyte vial into water bath until a few ice crystals are still remaining and clean exterior of the vial with 70% ethanol. Work quickly, complete the thawing process in less than 2 minutes.
3. In a biosafety hood, pour thawed cells into 15 mL empty conical tube, rinse the interior of the cryo vial with 1 mL of pre-warmed media from Tube A and bring volume of cell suspension plus media from Tube A to 14 mLs. Mix contents by inverting tube 2-3 times gently.
4. Spin the tube, at 60 x g, at room temperature for 7 min.
5. Aspirate supernatant, gently re-suspend pellet with solution from pre-warmed media from Tube B and bring volume to 12 mLs. Mix contents by inverting tube 2-3 times gently.
6. Centrifuge at 60 x g, room temperature for 3 min.
7. For plating re-suspend pellet with approximately 2 mLs of pre-warmed ISOM'S Seeding Media with 10% FBS, gently titrate cells 3 times with pipet. Measure the final volume for further calculation of total viable cells.

For suspension assays, resuspend human, monkey or dog CryoHepatocytes at 0.5×10^6 cells per mL in Williams E media. Resuspend rodent CryoHepatocytes in KBG buffer further supplemented with 1.8 mg/mL fructose (Sigma Cat. No. F-3510) and 0.225 mg/mL glycine (Sigma Cat. No. G-6388) at 0.5×10^6 cells/mL for rat or 0.25×10^6 cells/mL for mouse.

8. Keep thawed cells at room temperature and count immediately by following the next protocol; BD Gentest Determination of Cell Viability Protocol.