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Thawing of Plateable and Suspension Hepatocytes

Materials and Equipment:

- Water bath with heating
- Serological pipettes
- Micropipettes
- Centrifuge with rotor for 50 mL tubes
- +37 incubator with 5% CO_2
- Laminary hood
- 1 Vial of Cryopreserved Hepatocytes
- Nageotte counting chamber

Reagents:

- Hepatocyte Thawing Medium (20 mL)
- Additives for Thawing Medium (2.5 mL)
- Trypan Blue Solution
- Williams E Medium
- Antibiotic/Antimycotic (100x)

Caution!

It is critical to store obtained cells in the temperature lower then -150°C. DO NOT store cells in the dry ice. Use personal protective equipment: labcoat, gloves and goggles when working with cell cultures.

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Thawing of Human Hepatocytes

- Prepare thawing and culture medium by adding Additives for Thawing Medium (2.5 mL) to a 20 mL of Thawing Medium. Mix Williams E Medium with Antibiotic/Antimycotic Solution.
- Preheat Thawing Medium to 37°C before use and keep Williams E Medium at 4°C.
- Remove one cryovial with frozen Human Hepatocytes from the liquid nitrogen tank.
- Quickly place the cryovial in a 37°C water bath (do not use an incubator). Slightly open the vial to remove the excessive Liquid Nitrogen.
- While holding the tip of the bottle, mix gently, not allowing water to penetrate through the cap.

DO NOT submerge the cryotube completely in water.

- Thaw the vial of cells in a water bath until half of ice pellet remains (this usually takes about 80-115 seconds).
- Immediately remove the vial from the water bath. Wipe the outside of the bottle with an alcohol wipe. Place the thawed vial in the laminary hood.
- Transfer the contents of the cryovial to preheated Thawing Medium.
- Carefully rinse the cryotube with 1 ml of Thawing Medium few times and transfer the remaining cell suspension to a vial.
- Gently mix the cell suspension in the vial (DO NOT vortex)
- Centrifuge at 200g/ 6 min/ at room temperature
- Carefully aspirate the supernatant without disturbing the cell pellet.
- Resuspend the cell pellet in 3-4 ml of cold Cultivation medium. Do not use small tips to resuspend cells, it's better to use 5 mL pipette for more gentle resuspending of cell pellet.

- Measure the exact volume of the cell suspension with a 5 mL graduated pipette. Cell suspension should be stored on ice or at +4°C during all next procedures.
- Check cell concentration and viability by counting in chamber or using automated cell counter.

Write to hi@preci.care if you need any assistance. Online support is available for the best experimental results.