HEPG2 Subculturing in 100mm dish

1. Remove and discard culture medium.
2. Rinse cells with 3.5ml PBS and remove after 30 seconds to 1 minute
3. Add 1.5ml of Trypsin-EDTA solution to dish and place cells at 37 degrees to facilitate dissociation. Observe cells under an inverted microscope until cell layer is dispersed (usually within 3 to 8 minutes).
4. Add 3.5 mL of complete growth medium and collect cells in a 50ml conical tube.
5. Aseptically syringe cells 3x using a 5ml syringe and 22 gauge needle. **Discard both needle and syringe in sharps container. Do not disconnect needle from syringe, Do not recap needle.**
6. Add fresh complete growth media to new unused 100mm dishes
7. Add appropriate aliquots of the cell suspension to new dishes. (Recommended to use 1/2, 1/3, 1/4 split ratios)
8. Place cells in 37 degree incubator and shake in cross pattern to evenly distribute cells.

Preparing gelatin coated dishes for HEPG2

1. Retrieve and open new unused culture dishes
2. Add only enough 0.1% gelatin to cover the surface of the dish (100mm use ~3mls, 6 wells use ~0.5-1ml/well)
3. Place the gelatin coated dishes in a 37 degree incubator for 10 minutes
4. Aspirate the gelatin.
5. The dishes are now coated and ready for media/cells.