Isolation of Liver Tumor Cells from mice

(Xinhua Song and Yi Zhou, 2021)

Necessary supplies and equipment

70% EtOH

37℃water bath

Surgical tools (scissors, tweezers, and scalpels)

Collagenase (Sigma, C5138)

Solutions

1. 1X Washing buffer:

   Add 2.5 mM EGTA (0.475 g) to 500ml HBSS (without Calcium Chloride or Magnesium Chloride)

2. 1X HBSS-CaCl2 buffer:

   Add 0.706g $CaCl_2 2H_2O$ to 1000ml HBSS (without Calcium Chloride or Magnesium Chloride)

3. 100 mg stock collagenase solution:

   Dissolve 100mg collagenase (Sigma C5138) into 3.2 mL HBSS-$CaCl_2$ buffer. Next filter the solution through 0.45 µm pore filter, and store aliquots at -20℃.

4. 1X digestion buffer:

   Before beginning procedure, warm 1 mL of concentrated collagenase solution and dilute in 49 mL HBSS-$CaCl_2$ and warm to 37℃ before use.

   1ml: 49ml of HBSS-$CaCl_2$

   400ul:20ml

Liver tumor cells isolation:

1. Sac mice, rinse in 70% ethanol, dissect the liver tumor in the hood using autoclaved dissecting tools. Wash liver tissue with washing buffer in 10cm

plate.

2. Transfer the liver tumor (2-3g) to a new 10cm plate with washing buffer (1-2ml to ensure the liver tumor doesn't dry out) and mince the tumor into ~1mm fragments using scalpel blade.
3. Transfer the tumor fragments into 20ml digestion buffer in the 50ml falcon tube.
4. 37°C water bath for 15~30min until the medium becomes cloudy (gently vortex or revert for 2 secs per 5min), filter through 100 µm nylon mesh cell strainer into a new 50ml falcon tube.
5. Spin (700-1000rpm, 5 min). Remove the supernate and resuspend the cell pellet in 5 or 10ml DMEM containing 10% FBS and 1% Penicillin-Streptomycin, then transfer into 5 or 10cm plate.
6. Change the medium every 3 days. The cell types are mixed at the beginning. Non-tumor cells will be gradually eliminated by tumor cells. Passage cells when the cells are uniform (2~4 weeks).