Isolation of primary hepatoblasts

Protocol adapted from Tanimizu N et al., J Cell Sci. 2003 and Kamiya A et al, EMBO J 1999

1) Remove the livers from E14.5 embryos

- Digestion of the embryonic livers:

2) Place embryonic livers into prewarmed Liver Perfusion Medium (LPM) (Invitrogen, No.17701) (1.5 mL medium per liver).
3) Break livers mechanically by pipeting the tissue in LPM 3-5 times through a glass pipette.
4) Incubate livers in LPM for 20 min at 37 ºC waterbath, mix with turning after every 5 min.
5) Centrifuge at 212×g, 3 min at room temperature.
6) Remove supernatant and loosen the pellet (necessary for avoiding aggregation).
7) Suspended liver pieces in 12ml Liver Digest Medium (LDM) (Invitrogen, No.17703-034) supplemented with DNase I (1:1000).
8) Incubate liver pieces in LDM for 20 minutes at 37 ºC, pipetting every 5 minutes.
9) Drain cell suspension through a cell strainer (70 μm) (BD Falcon, No.352350). Add PBS until 40 mL of cell suspension.
10) Centrifuge at 260×g, 3 min at room temperature.
11) Remove the supernatant and loosen pellet to avoid aggregation.
12) Resuspend cells with 10 mL of PBS.
13) Drain the suspension through cell strainer (70 μm) again and wash the tube and the cell strainer with additional 30 mL of PBS.

- Hemolysis

14) Centrifuge at 260×g for 3 min at room temperature.
15) Remove supernatant and loosen pellet.
16) Add 12 mL of ice-cold Hemolysis buffer (12 mL per 8 livers) and resuspend the pellet.
17) Incubate on ice for 5 min.
18) Gently add 23 mL ice-cold Hepatoblasts Differentiation Medium (HDM) containing 5% FBS. Remove debris by passing suspension through 70 μm cell strainer.
19) Wash the walls of the tube with additional 5 mL of ice-cold HDM and pass the solution through the cell strainer.
20) Centrifuge at 260×g for 3 min at room temperature.
21) Remove supernatant and loosen pellet.
22) Resuspend cells with 10 mL ice-cold HDM and pass through the cell strainer.
23) Wash the tube again with 30 mL PBS and drain through cell strainer.
24) Count the number of cells.
25) Centrifuge at 260×g for 3 min at room temperature.
26) Remove supernatant and loosen pellet.
27) Resuspend cells (100,000,000 cells / mL) into MACS buffer and split the cell suspension between eppendorfs (1.5 mL), 300 μL of cells in MACS buffer into one eppendorf.

- Magnetic cell sorting

28) Incubate cells with anti-FcgR antibody (1:100 dilution) on ice for 10 min.
29) Add FITC anti-Dlk1 antibody (1:40 dilution) and incubate on ice for 15 min.
30) Add 1 mL MACS buffer and centrifuge at 660×g (tabletop centrifuge) for 3 min at 4ºC.
31) Remove supernatant and loosen pellet.
32) Wash again with 1 mL MACS buffer and centrifuge at 660×g (tabletop centrifuge) for 3 min at 4ºC .

33) Remove supernatant and loosen pellet.
34) Resuspend cells with 150 μl MACS buffer / 30,000,000 cells and add anti-FITC-microbeads (1:10 dilution). Incubate on ice for 15 minutes.
35) Add 1 mL MACS buffer and centrifuge at 660×g (tabletop centrifuge) for 3 min at 4ºC.
36) Remove supernatant and loosen pellet.
37) Resuspend cells in 3 mL MACS buffer. Remove debris by passing through a cell strainer (70 μm) into a 15 mL tube.
38) Centrifuge at 260×g for 3 min at room temperature.
39) Remove supernatant and loosen pellet.
40) Resuspend in 500 μl of MACS buffer (up to 100,000,000 cells can be passed through the MACS column).
41) Install a MACS column (MS column, Miltenyi Biotech, No.130-042-201) onto a magnetic holder and equilibrate it by washing through 500 μl MACS buffer.
42) Load the 500 μl cell suspension onto the MACS column and collect flow.
43) Load again.
44) Wash column 3 times with 500 μl MACS buffer.
45) Take MACS column off magnetic holder.
46) Put MACS column onto a 15 mL tube and elute Dlk1-positive cells with 3x 1 mL MACS buffer.
47) Count the number of cells.
48) Centrifuge at 260×g for 3 min at room temperature.
49) Remove supernatant and loosen pellet.
50) Resuspend cells in icecold HDM (1,000,000 cells / mL)

Hepatocyte differentiation medium:

- DMEM (High glucose 4.5 g/mL)
- 1x Pen/Str
- 1x L-glutamine (Invitrogen, No. 25030-024)
- 1x non-essential amino acids (Invitrogen, No. 11140-035)
- Dexamethasone 31.25 µL per 500 mL of medium (Sigma-Aldrich, D1756-25MG)
- Remove 25 mL of sf medium
- add 25 mL of filtered serum (this serum is not heat-inactivated!) (filter and then immediately wash the filter with some medium to get all serum proteins)

MACS buffer:

- 0.5% BSA
- 2 mM EDTA
- PBS
- filter the buffer before use!