Primary Human Liver Dissociation for scSeq

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Abstract

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Guidelines

Reagents to prepare for dissociation:

- 4 tissue dissociator tubes
- 4 falcon tube with 20ml Percoll solution 25% in PBS minus $Ca^{+2}Mg^{+2}$ (cold)
- 4 falcon tubes with Miltenyi Biotech Debris Removal Solution
- 1 bottle of PBS (-)$Ca^{+2}Mg^{+2}$ (warm)
- 100ml PBS (-)$Ca^{+2}Mg^{+2}$ (+)EDTA 0.5mM (warm)
- 100ml PBS (+)$Ca^{+2}Mg^{+2}$ (filtered and at 37°C)
- L**iberase enzyme solution** - 5.2 mg/ml stock (Sigma 000000005466202001)
  0.2 Wünsch units/ml (0.385ml stock + 9.615ml Hepatozyme media)
- **Stop solution**: 20% FBS in PBS (-)$Ca^{++}Mg^{++}$ (cold)
- **Red blood cell lysis buffer**: 1:10 dilution of Miltenyi Biotec RBC lysis buffer in ddH2O (MilliQ water)
- **Miltenyi Biotech debris removal solution**
- **Hepatozyme media (-)growth factors (cold)**

Thumbnail image:

https://www.tutorialspoint.com/digestion_and_absorption/digestive_glands_liver.asp

Materials

Please see Guidelines for required materials. by Contributed by users

Protocol

Step 1.
Wash bulk liver sample in a 50 ml tube with **warm PBS (-)$Ca^{+2}Mg^{+2}$ (+)EDTA**. (1/2)

Step 2.

Wash bulk liver sample in a 50 ml tube with **warm PBS (-)$Ca^{+2}Mg^{+2}$ (+)EDTA**. (2/2)

Step 3.

Distribute liver into even pieces 2-3g each.

Step 4.

Dice into small pieces in a petri dish with **warm PBS (-)$Ca^{+2}Mg^{+2}$ (+)EDTA**.

Step 5.

Then transfer into tissue dissociator tubes.

Step 6.
Wash samples with **warm (37°C) PBS (+)$Ca^{+2}Mg^{+2}$** to activate collagenase. (1/2)

Step 7.

Wash samples with **warm (37°C) PBS (+)$Ca^{+2}Mg^{+2}$** to activate collagenase. (2/2)

Step 8.

Add warm 0.2 Wünsch units/ml Liberase collagenase solution to tissue samples.

Step 9.

Incubate at 37°C, 30 minutes on shaker

TEMPERATURE
37 °C : Incubation on shaker at 200rpm

DURATION
00:30:00 : Incubation

Step 10.

Process each tube containing tissue through 2 “B” cycles using the “C tube” specific protocol of the Miltenyi Biotech GentleMACS tissue dissociator.

Step 11.

Filter each digestion through a 70 µm filter into its own respective 50 ml falcon tube - use the back of a plunger to push the filtrate through.

Step 12.

Add a 1:1 ratio of 20% FBS stop solution to the collected cell suspension and wash the filter; 10 ml stop solution.

Purification procedure for hepatocyte fraction

Step 13.

Centrifuge cell suspensions at 50g, 5 min, 4°C to pellet the hepatocyte fraction.

TEMPERATURE
4 °C : Centrifugation
DURATION
00:05:00 : Centrifugation

Purification procedure for hepatocyte fraction

Step 14.

Collect the NPC-containing supernatant in a separate tube and place on ice for later purification.

Purification procedure for hepatocyte fraction

Step 15.

Resuspend each hepatocyte pellet in **5 ml of PBS**.

AMOUNT
5 ml : PBS

Purification procedure for hepatocyte fraction

Step 16.

Add 5 ml of the cell suspension carefully on top of four separate cold 25% percoll solution

(25 ml final volume).

AMOUNT
5 ml : Cell suspension

Purification procedure for hepatocyte fraction

Step 17.

Centrifuge the percoll gradients at 1250g, 20 mins, 4°C without brake.

TEMPERATURE
4 °C : Centrifugation
DURATION

00:20:00 : Centrifugation

Purification procedure for hepatocyte fraction

Step 18.

Aspirate percoll and debris; hepatocytes should pellet at the bottom, while debris should be floating on the top of the gradient.

Purification procedure for hepatocyte fraction

Step 19.

Resuspend each hepatocyte pellet in 2 ml of **RT RBC lysis buffer** and incubate at RT for 7-10 minutes.

AMOUNT

2 ml : RT RBC lysis buffer

DURATION

00:07:00 : Incubation

Purification procedure for hepatocyte fraction

Step 20.

Centrifuge 50g, 5 mins, 4°C and aspirate the red supernatant.

TEMPERATURE

4 °C : Centrifugation

DURATION

00:05:00 : Centrifugation

NOTES

The pellet should be light brown in color.

Purification procedure for hepatocyte fraction

Step 21.

Resuspend hepatocyte pellets (total/pooled) in 1 ml total **cold Hepatozyme media (-)GF**.

AMOUNT

1 ml : Cold Hepatozyme media (-)GF

Purification procedure for hepatocyte fraction

Step 22.

Count cells and dilute to the appropriate concentration using Hepatozyme media.

Store on ice.

Purification procedure for NPC fraction

Step 23.

Centrifuge the NPC-containing supernatants at 300g, 5 min, 4°C (1^{st} NPC fraction).

TEMPERATURE

4 °C : Centrifugation

DURATION

00:05:00 : Centrifugation

Purification procedure for NPC fraction

Step 24.

Resuspend the pellet in **3.1 ml of PBS**.

AMOUNT

3.1 ml : PBS

Purification procedure for NPC fraction

Step 25.

Centrifuge supernatant at 650g, 7 min, 4°C (2nd NPC fraction).

TEMPERATURE

4 °C : Centrifugation

DURATION

00:07:00 : Centrifugation

Purification procedure for NPC fraction

Step 26.

Resuspend the pellet with the **3.1 ml of PBS** from the 1st NPC fraction.

Purification procedure for NPC fraction

Step 27.

Add 900 µl of debris removal solution to the pooled NPC suspension.

AMOUNT

900 µl : Debris removal solution

Purification procedure for NPC fraction

Step 28.

Follow the Miltenyi Biotech Debris Removal protocol to produce a clean cell pellet.

Purification procedure for NPC fraction

Step 29.

Resuspend each NPC pellet in 2 ml of **RT RBC lysis buffer.**

AMOUNT

2 ml : RT RBC lysis buffer

Purification procedure for NPC fraction

Step 30.

Incubate resuspended NPC pellet at RT for 10 minutes.

DURATION

00:10:00 : Incubation

Purification procedure for NPC fraction

Step 31.

Centrifuge at 650g, 5 min, 4°C to pellet cells.

TEMPERATURE

4 °C : Centrifugation

DURATION

00:05:00 : Centrifugation

Purification procedure for NPC fraction

Step 32.

Aspirate red supernatant and resuspend cell pellet in 1 ml of **cold Hepatozyme media (-)GF**.

AMOUNT

1 ml : Cold Hepatozyme media (-)GF

Purification procedure for NPC fraction

Step 33.

Count cells and dilute to the appropriate concentration using Hepatozyme media. Store on ice.