ZerialLab

MAX PLANCK INSTITUTE
OF MOLECULAR CELL BIOLOGY
AND GENETICS

Primary Mouse Hepatocyte Isolation

Adapted from: Hengstler et al. Arch Toxicol (2008) 82:923–931; DOI 10.1007/s00204-008-0375-x
Reference: Zeigerer et al. Exp Cell Res (2017) Jan 1;350(1):242-252; DOI: 10.1016/j.yexcr.2016.11.027.

Note: Local authorities must approve all animal experiments beforehand!

Materials

- Glucose solution (9 g/l)
- KH buffer (60 g/l NaCl, 1.75 g/l KCl, 1.6 g/l KH_2PO_4, pH adjusted with NaOH to 7.4)
- HEPES buffer (60 g/l HEPES, pH adjusted with NaOH to 8.5)
- Amino acid solution
    - 0.27 g/l l-Alanine, 0.14 g/l L-Aspartic acid, 0.4 g/l Asparagine, 0.27 g/l Citrulline, 0.14 g/l L-Cysteine, 1 g/l L-Histidine, 1 g/l L-Glutamic acid, 1 g/l L-Glycine, 0.4 g/l L-Isoleucine, 0.8 g/l L-Leucine, 1.3 g/l L-Lysine, 0.55 g/l L-Methionine, 0.65 g/l L-Ornithine, 0.55 g/l L-Phenylalanine, 0.55 g/l L-Proline, 0.65 g/l L-Serine, 1.35 g/l L-Threonine, 0.65 g/l L-Tryptophan, 0.55 g/l L-Tyrosine, 0.8 g/l L-Valine
    - Amino acids, which cannot be resolved at neutral pH, can be solubilized using 10N NaOH; adjust the pH afterwards with HCl to 7.6
- Glutamine solution (7 g/l)
- $CaCl_2$ solution (19 g/l $CaCl_2*2H_2O$)
- $MgSO_4$ solution (24.6 g/l $MgSO_4*7H_2O$)
- EGTA solution (47.5 g/l EGTA and 10 M NaOH, adjust pH to 7.6)

➔ All solutions have to be sterile filtered and stored at 4 °C. Amino acid solutions should be aliquoted and stored at -20 °C.
➔ Glutamine and EGTA solution should be stored no longer than 2 months.

EGTA buffer:

124 ml Glucose solution
20 ml KH buffer
20 ml HEPES buffer
30 ml amino acid solution
2 ml Glutamine solution
0.8 ml EGTA solution

Collagenase buffer:

155 ml Glucose solution
25 ml KH buffer
25 ml HEPES buffer
38 ml amino acid solution
10 ml $CaCl_2$ solution
2.5 ml Glutamine solution
Add the collagenase into the collagenase buffer during the perfusion step with EGTA
Per bottle collagenase buffer use ~90 mg (300-600 units)

Suspension buffer:

124 ml Glucose
20 ml KH buffer
20 ml HEPES buffer
30 ml amino acid solution
2 ml Glutamine solution
1.6 ml $CaCl_2$ solution
0.8 ml $MgSO_4$ solution
400 mg BSA

Method

Prepare EGTA, collagenase and suspension buffer fresh directly before the isolation in a sterile hood.
Prewarm buffers to 37 °C in a water bath.
Precool centrifuge to 4 °C.

Prepare a peristaltic pump with tube and needle (20G x 1 ½", 0.9x40mm, not sharp). The flow rate should be 10 ml/min. Rinse with EGTA buffer to check for any air bubbles before the perfusion starts.

The mouse is anaesthetized using an intraperitoneal injection with Ketamin:Rompun (1.6:1). Make sure that all reflexes are fully gone before starting. Fix mouse onto a sterile surface.

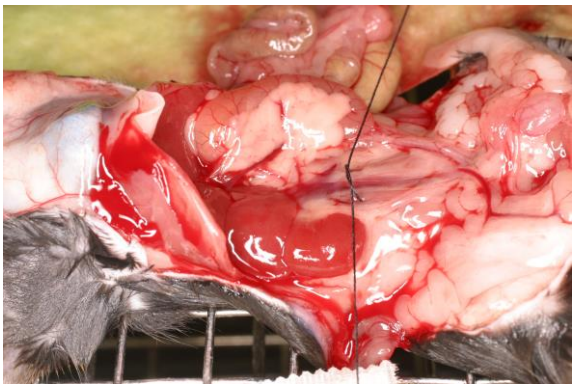
Carefully spray the mouse with EtOH. Open the upper abdominal wall using a surgical scissor. Start cutting into the skin around the height of the bladder and remove the skin until reaching the diaphragm. Make sure not to injure the lower abdominal wall.

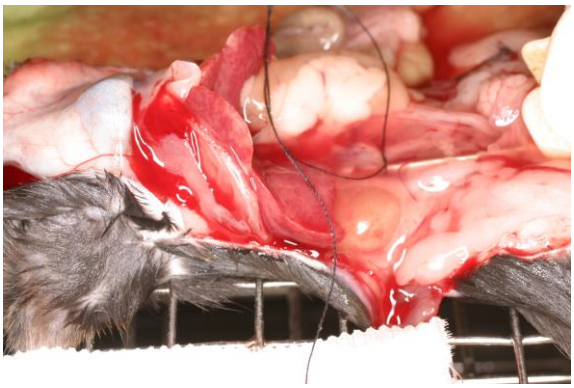
Now cut into the left side of the upper skin as shown in the picture (green arrow), so that the perfusion solutions can drain into the supplied container. Clean the lower abdominal wall with 1x sterile PBS to remove all remaining hair.

Remove the lower abdominal wall using a fine surgical scissor. Be careful not to injure any organs.

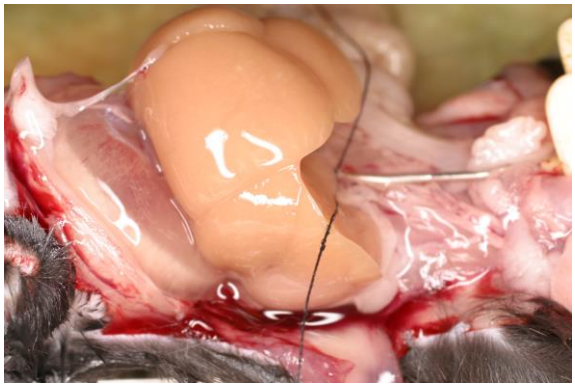
Place a 1x sterile PBS-soaked tissue on the right side of the mouse. Carefully move the intestine and fat tissue to expose the *venae cavae* and the hepatic portal vein. Gently lift the liver lobules carefully against the thorax.

Use fine forceps to place a piece of thin thread below the *venae cavae.* Tie a loose knot that will later secure the needle inside the *venae cavae.* Use fine surgical scissor to make a nick in the *venae cavae* – do not fully cut it! Start the peristaltic pump and insert the needle into this opening. If placed correctly the liver will quickly loose its red color. Immediately cut the hepatic portal vein to drain the solutions. If done too late high pressure will damage the hepatocytes leading to reduced viability of the cells. Rinse the liver for 10 min with EGTA buffer. Place a warm red light lamp close to the mouse.

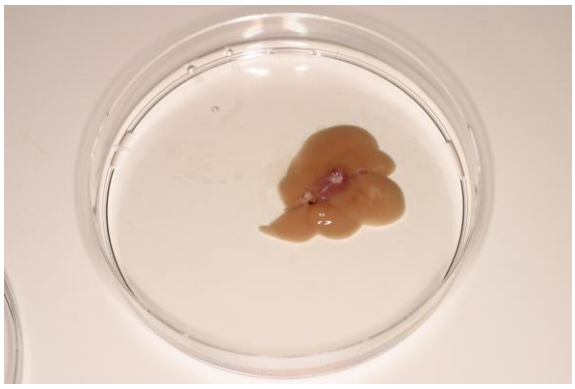




After 5 min prepare ~90 mg collagenase (on ice) and put it into the collagenase buffer. Place it back into the water bath. When the time is up stop the peristaltic pump and switch the tube into the collagenase solution. Rinse for 8 min (do not forget to put on the warm red light lamp!). The liver should get big and soft.



After the perfusion, prep the liver carefully out of the animal. Using forceps grab into the diaphragm and cut with a small scissor the surrounding tissue. Be careful not to harm the intestine. Place the liver into a petri dish filled with app. 15 ml suspension buffer.



Bring now the liver, the suspension buffer and two small forceps into a sterile hood and extract the hepatocytes out of the liver using the two forceps. Thereby grab the liver only with one forceps on the remaining part of the diaphragm and use the other forceps to rip carefully through the soft liver tissue. The hepatocytes will “fall out” from the liver with soft swinging of the tissue. Do not use any force, since this could damage the hepatocytes.

The resulting hepatocyte suspension will now be filtered through a cell strainer (pore size of 100 um) into a 50 ml tube. Fill the tube to 50 ml with suspension buffer. Centrifugation for 5 min at 50x g at 4°C. Wash the obtained cell pellet with another 50 ml suspension buffer and centrifuge solution as before. Resuspend the resulting pellet in 10 ml suspension buffer by gently inversion.

After isolation, the cells should be kept on ice. They can be stored on ice for up to 20 hours, but the viability drops significantly. If the hepatocytes are to be shipped or stored, it is better to fill the falcon tube completely (50 ml) with resuspension buffer, to minimize air in the tube.
For later use, spin the cells down again with 50x g for 5 min and resuspend the cells in 10 ml buffer again.

Determine cell number and vitality of the prep by using Trypan blue exclusion procedures:
Dilute 100 µl of the cell suspension (make sure to invert the tube gently before taking an aliquot to dissolve all cells; use a cut-off tip) into 400 µl of resuspension buffer. Take 50 µl and mix this in 50 µl of Trypan blue solution. Apply to the Neubauer counting chamber and count all four squares.

To determine vitality, count dead cells and live cells separately. Calculate the concentration by using the following equation: mean number of live cells/square x 10 000 x dilution factor = number of cells/ml.

Usually the prep of one BL6 male mouse (8 to 12 weeks old) results in total of 30 to 100 mio cells. Cells with a vitality of < 75 % should not be used.