Mouse Hepatic Parenchymal Cell Isolation and Culture Kit

Cat.No. : P-CA-707

Size：3Tests/10Tests

Background

This kit is based on an improved classical perfusion digestion method for isolating and extracting mouse hepatic parenchymal cells. After each experiment (per T), at least 1 × 10^7 mouse hepatic parenchymal cells can be obtained, following the perfusion digestion of liver tissue from one mouse, as well as the isolation and purification. Immunofluorescence identification shows that the cell purity (CK-18 positive rate) is > 90%.

Scope of Application

This product is suitable for Kunming, C57BL/6, BALB/c and other different species of mice aged 28-84 days. After perfusing and digesting the liver tissue, performing low-speed centrifugation, using trypan blue staining and cell counting, a yield of >1 × 10^7 cells can be obtained with the cell purity (CK-18 positive rate) exceeding 90%. The obtained hepatocytes continue to differentiate after 24 hours of cultivation, gradually losing their morphological characteristics, and the entire cultivation cycle can last for 1-2 weeks.

Components of Kit

The components of this kit is shown in the table below

Table 1. Composition and corresponding information of the Kit

Name	Size	Appearance	Storage and Expiration Date
Pre-perfusion Solution For Mouse Hepatic Parenchymal Cells	3Tests (125 mL) 10Tests (400 mL)	Colorless Clear Liquid	2-8°C, 1 year
Diluent Of Perfusion Digestive Enzymes For Mouse Hepatic Parenchymal Cells	3Tests (125 mL) 10Tests (400 mL)	Red Clear Liquid	2-8°C, 1 year
Perfusion Digestive Enzyme For Mouse Hepatic Parenchymal Cells	3Tests (600 μL) 10Tests (2 mL)	Brown Clear Liquid	-5~-20°C, 1 year
Specialized Washing Solution For Mouse Hepatic Parenchymal Cells	3Tests (250 mL) 10Tests (500 mL)	Red Clear Liquid	2-8°C, 1 year
Specialized Isolation Solution For Mouse Hepatic Parenchymal Cells	3Tests (75 mL) 10Tests (250 mL)	Colorless Clear Liquid	2-8°C, 1 year
Coating Buffer For Mouse Hepatic Parenchymal Cells	3Tests (20 mL) 10Tests (50mL)	Colorless Clear Liquid	2-8°C, 1 year
Basic Culture Medium A For Mouse Hepatic Parenchymal Cells	3Tests (75 mL) 10Tests (250 mL)	Red Clear Liquid	2-8°C, 1 year
Supplement A For Mouse Hepatic Parenchymal Cells	3Tests (7.5 mL) 10Tests (25 mL)	Yellow Clear Liquid	-5~-20°C, 1 year

Continued

Name	Size	Appearance	Storage and Expiration Date
Basic Culture Medium B For Mouse Hepatic Parenchymal Cells	3Tests (150 mL) 10Tests (500 mL)	Red Clear Liquid	2-8°C, 1 year
Supplement B For Mouse Hepatic Parenchymal Cells	3Tests (15 mL) 10Tests (50 mL)	Red Clear Liquid	-5~-20°C, 1 year
70 μm Cell Filter	3Tests (3 pcs) 10Tests (10 pcs)	Orange	Room temperature, 3 years

Note: Please store each component at the temperature indicated on the label of the reagent tube. After thawing, the digestive fluid should be stored at 4°C for 30 days. Following the first use of the kit, it is recommended to aliquot the digestive fluid into single-use portions according to the instruction and store at a -20°C refrigerator. Thaw again before use to avoid repeated freezing and thawing.

Notes

1. In this experiment, you need to prepare PBS buffer, trypan blue staining solution, syringes, scissors, forceps, culture vessels, venous indwelling needle, and other reagents and consumables. If the cultivation needs to be expanded, please prepare complete medium independently.
2. If the isolation process of primary hepatocytes is not handled properly, contamination can occur easily. Ensure that all equipment used in the experiment is sterile.
3. Primary hepatocytes cannot be passaged. Do not use various digestive enzymes to digest and passage the fully confluent hepatocytes after extraction.
4. Hepatocytes are sensitive to fluid shear forces. During the isolation process, avoid vortexing or excessive pipetting when resuspend the cell pallet, in order not to affect cell viability.
5. Liver tissue perfusion can be performed by peristaltic pumps, infusion machines and manual operation. During the process, pay attention to controlling the perfusion flow rate to avoid incomplete or excessive tissue digestion. The following steps take peristaltic pumps and manual perfusion as examples, and the perfusion method can be adjusted according to specific laboratory conditions.
6. The operation of mouse liver perfusion digestion is complicated. Prior to formal experiments, it is recommended to practice pre-perfusion to familiarize yourself with operational procedures and avoid unnecessary waste.
7. Please note that during storage, the Specialized Isolation Solution For Mouse Hepatic Parenchymal Cells in this kit may exhibit crystallization of particulate matter at the bottle mouth or cloudiness in the solution. These are normal phenomenon and the product can be safely used.
8. Due to the bloody process of mouse perfusion, this manual does not provide actual photos for reference, but only schematic diagram. If necessary, please contact the technical support to obtain actual photos of the experimental process.

Operational Procedures

1. Pre-experiment Preparation

1) Preparation of Culture Medium: Prepare Adhesion Culture Medium For Mouse Hepatic Parenchymal Cells and Maintenance Medium For Mouse Hepatic Parenchymal Cells according to the experimental dosage.
   ① Adhesion Culture Medium For Mouse Hepatic Parenchymal Cells
   Basic Culture Medium A For Mouse Hepatic Parenchymal Cells: Supplement A For Mouse Hepatic Parenchymal Cells=10: 1.
   ② Maintenance Medium For Mouse Hepatic Parenchymal Cells
   Basic Culture Medium B For Mouse Hepatic Parenchymal Cells: Supplement B For Mouse Hepatic Parenchymal Cells=10: 1.
   Note:Adhesion and maintenance culture medium: 2–8°C, valid for 3 months. When preparing above-mentioned culture medium, it can be prepared according to the usage amount. Remaining supplements should be aliquoted proportionally and stored at -20°C to avoid repeated freeze-thaw cycles.
2) Self-supplied Reagents and Consumables: dissection board (foam board can substitute), ice plate or ice box, surgical instruments (at least 2 pairs of ophthalmic scissors, 3 pairs of straight forceps, 2 pairs of curved forceps, 2 pairs of microscopic forceps, hemostatic clips), 6 cm culture dishes, and several 15 mL/50 mL centrifuge tubes.
3) Preparation of Perfusion Digestive Working Solution For Mouse Hepatic Parenchymal Cells: Thaw Perfusion Digestive Enzyme For Mouse Hepatic Parenchymal Cells at 4°C. Open the Diluent Of Perfusion Digestive Enzymes For Mouse Hepatic Parenchymal Cells in a clean bench. Add 100 µL of Perfusion Digestive Enzyme For Mouse Hepatic Parenchymal Cells into 20 mL of Diluent Of Perfusion Digestive Enzymes For Mouse Hepatic Parenchymal Cells, mix thoroughly.
4) Before the experiment, equilibrate the Pre-perfusion Solution For Mouse Hepatic Parenchymal Cells and Perfusion Digestive Working Solution For Mouse Hepatic Parenchymal Cells in a 37°C water bath for about 30 min.
5) Coat the culture dish with Coating Buffer For Mouse Hepatic Parenchymal Cells. Incubate at 37°C for more than 2 hours, or coat overnight at 4°C.

2. Mouse Perfusion Digestion

1) Anesthesia and Fixation of Mice: After anesthetizing mice with anesthetics, spray the mice all over with 75% alcohol. Transfer them to a super clean bench, and secure the mice in a supine position on the dissection board with needles.
2) Mouse Anatomy:
   ① Preparation: Place two clean culture plates in the ultra clean bench. Arrange sterilized scissors and forceps in pairs from left to right on two clean culture plates (Ophthalmic Scissors 1 and Straight Forceps 1, Ophthalmic Scissors 2 and Straight Forceps 2, Curved Forceps 1, Curved Forceps 2, and Curved Forceps 3).
   Note: The distal third of the instruments should extend beyond the plates to avoid contamination. After each use, return tools to their original positions and make sure they don't touch each other to prevent cross-contamination.
   ② Dissection: First, incise the mouse skin along the midline of the abdomen with Ophthalmic Scissors 1. Lift the skin along the incision site with Straight Forceps 1, and use Ophthalmic Scissors 1 to cut open the skin horizontally towards the limbs. Bluntly dissect the skin and subcutaneous tissue toward both sides to expose the superficial muscle layer of the abdominal wall. Continue to use Straight Forceps 1 to pull the skin to both sides and peel it

down below the abdomen. Then use Ophthalmic Scissors 2 to incise the diaphragm of the mouse abdomen, and use Straight Forceps 2 to gently retract the diaphragm down below the abdomen.

Note: (Optional Operation) After incising the abdominal diaphragm, proceed to incise the thoracic diaphragm along the upper edge of the liver. Find the inferior vena cava above the liver and clamp the vein at this site with a hemostatic clip (Figure 1). This step allows the subsequent digestive solution to fully flow through the liver tissue and exit through the hepatic portal vein, improving digestion efficiency. If it is not possible to accurately locate the inferior vena cava above the liver and promptly complete the subsequent steps, please ignore this step.

3) Mouse perfusion: The mouse perfusion method depends on the specific laboratory conditions. If peristaltic pump perfusion is used, please follow steps a - c. If manual perfusion is used, please follow steps d - g.

① **Peristaltic Pump Perfusion**

a. Lift up the outer lobe of the mouse liver using Curved Forceps 1 and push the intestinal tissue to the right, exposing the inferior vena cava and hepatic portal vein of the mouse (a pipette tip can be placed under the mouse to fully expose the inferior vena cava). Take a perfusion needle (exhaust all air) and connect the inlet end of it to a centrifuge tube or botttle containing Pre-perfusion Solution For Mouse Hepatic Parenchymal Cells. The needle should be parallel to the inferior vena cava, with the oblique plane of the needle pointing upwards. Insert it into the inferior vena cava (the injection position is shown in Figure 1), and insert the needle tip above the bifurcation of the blood vessel. Fix the perfusion needle and use other auxiliary materials (such as syringe needles) to keep the needle still and avoid puncturing the blood vessel. Gently pull out the needle core, then the blood in the blood vessel can be seen to flow out with the needle core. At this time, the outer tube is left in the blood vessel for perfusion.

b. Turn on the peristaltic pump and slowly infuse the Pre-perfusion Solution For Mouse Hepatic Parenchymal Cells until the liver becomes fully distended. At this time, use Ophthalmic Scissors 2 to cut open the hepatic portal vein for drainage (marked in Figure 2), maintaining a perfusion rate at 5 mL/min until all blood in the liver are completely drained and the liver tissue turns waxy yellow, the perfusion volume is about 20 mL (the perfusion process is shown in Figure 3).

Note: In this step, all liver lobes should turn waxy yellow. If there is blood stasis, gently remove it with Curved Forceps 1. If only a portion of the liver lobe become waxy yellow or appear large areas of blood color that cannot be dispersed, it indicates perfusion failure. The specific reasons are detailed in Table 2 below.

c. Transfer the inlet end of the hose into a centrifuge tube or bottle containing 37°C preheated Perfusion Digestive Working Solution For Mouse Hepatic Parenchymal Cells, avoiding air entering the tube. Turn on the peristaltic pump and continue perfusion digestion, maintaining a flow rate at 5 mL/min. During the perfusion process, rhythmically squeeze the hepatic portal vein incision with Curved Forceps 1 (squeeze for about 3-5 seconds, with an interval of 0.5-1 min, as to fill the liver and improve digestion rate). The perfusion time varies from 5-8 minutes. When the liver tissue exhibits a honeycomb-like punctate pattern, demonstrates slow rebound after gentle pressure, and displays cracked striations on the surface, it indicates the completion of digestion.

② **Manual Perfusion**

d. Use two 50 mL syringes to separately aspirate the preheated Pre-perfusion Solution For Mouse

Hepatic Parenchymal Cells and Perfusion Digestive Working Solution For Mouse Hepatic Parenchymal Cells. Remove the needle and connect the hub of venous indwelling needle to the tip of the syringe containing the Pre-perfusion Solution, ensure that it is stable with no leakage. Expel all air from the syringe and needle tube, then set aside for later use.

e. Lift up the outer lobe of the mouse liver using Curved Forceps 1 and push the intestinal tissue to the right, exposing the inferior vena cava and hepatic portal vein of the mouse (a pipette tip can be placed under the mouse to fully expose the inferior vena cava). Take the perfusion needle (exhaust all air) which has been connected to the syringe containing Pre-perfusion Solution For Mouse Hepatic Parenchymal Cells. The needle should be parallel to the inferior vena cava, with the oblique plane of the needle pointing upwards. Insert it into the inferior vena cava (the injection position is shown in Figure 1), and insert the needle tip above the bifurcation of the blood vessel. Fix the perfusion needle and use other auxiliary materials (such as syringe needles) to keep the needle still and avoid puncturing the blood vessel. Gently pull out the needle core, then the blood in the blood vessel can be seen to flow out with the needle core. At this time, the outer tube is left in the blood vessel for perfusion.

f. Gently push the syringe to allow the Pre-perfusion Solution into the mouse liver tissue. Use a timer to start the timing, control the infusion rate, and maintain the flow rate at around 5 mL/min. When the liver is full, use Ophthalmic Scissors 2 to cut open the hepatic portal vein for drainage (marked in Figure 2). Keep perfusion until the blood in the liver are completely drained and the liver tissue turns waxy yellow, the whole perfusion time is about 4-5 minutes (the perfusion process is shown in Figure 3).

Note: In this step, all liver lobes should turn waxy yellow. If there is blood stasis, gently remove it with Curved Forceps 1. If only a portion of the liver lobe become waxy yellow or appear large areas of blood color that cannot be dispersed, it indicates perfusion failure. The specific reasons are detailed in Table 2.

g. Grasp the needle holder of the indwelling needle, remove the syringe, and connect the hip of the indwelling needle to another syringe containing Perfusion Digestive Working Solution For Mouse Hepatic Parenchymal Cells. Continue perfusion, start the timer, control the flow rate at 3-5 mL/min, and rhythmically squeeze the hepatic portal vein incision with Curved Forceps 1 during the perfusion process (squeeze for about 3-5 seconds, with an interval of 0.5-1 min to fill the liver and improve digestion rate). Each mouse needs 30-40 mL of Perfusion Digestive Working Solution. When the liver tissue exhibits a honeycomb-like punctate pattern, demonstrates slow rebound after gentle pressure, and displays cracked striations on the surface, it indicates the completion of digestion.

4) Cut the vascular and connective tissue connected to the liver along the diaphragm. Gently lift the liver tissue with Curved Forceps 2, cut off the remaining connecting tissue. Transfer the intact liver tissue to a pre-prepared culture dish containing Specialized Washing Solution For Mouse Hepatic Parenchymal Cells. Secure the liver tissue at the junction of the liver lobes using Curved Forceps 2 in a super clean bench. After removing the gallbladder with Curved Forceps 3, gently peel off the hepatic capsule. Hepatic parenchymal cells can be seen flowing out like quicksand, when the tissue is gently shaked. The incomplete dispersed parts can be gently separated with forceps and dispersed into the cell washing solution (Figure 4).

3. Isolation and Purification of Hepatocytes

1) Place a 70 μm cell filter over the mouth of a brand new 50 mL centrifuge tube, add 1-2 mL of

Specialized Washing Solution For Mouse Hepatic Parenchymal Cells to moisten the filter. Use a 5 mL pipette to aspirate the cell suspension in the culture dish obtained from the previous steps and transfer it to the top of the cell filter for filtration. After complete filtration, add 5 mL of Washing Solution to rinse the bottom of the dish, aspirate the rinsing solution and filter.

2) Then transfer the filtrate into a 15 mL centrifuge tube. Centrifuge 50 g for 5 minutes.
3) Discard the supernatant and add 20 mL of Specialized Washing Solution For Mouse Hepatic Parenchymal Cells to resuspend the cell pellet. Gently shake the centrifuge tube and then pipette the cell suspention up and down to evenly disperse the cells in the solution.
4) Repeat step (2).
5) Discard the supernatant and add 10 mL of Specialized Washing Solution For Mouse Hepatic Parenchymal Cells to resuspend the cell pellet. Gently shake the centrifuge tube and then pipette the cell suspention up and down to evenly disperse the cells in the solution.
6) After gently shaking, immediately aspirate a small amount of cell suspension, stain with trypan blue staining solution, and observe cell viability under a inverted microscope (it is not recommended to use a regular counter for counting, as there is a large deviation from the actual results). If the cell viability is above 40%, subsequent purification experiments can be carried out. If the viability is low, it may be due to excessive digestion and perfusion, and it is not recommended to continue the experiment. The specific reasons can refer to Table 2.
7) Add an equal amount of Specialized Isolation Solution For Mouse Hepatic Parenchymal Cells into four 15 mL centrifuge tubes, with 5 mL per tube. Gently layer the cell suspension along the wall of the tube onto the cell isolation solution to form a distinct interface between the two solutions.
8) Centrifuge at 400 g for 10 minutes (using a horizontal rotor with the lowest acceleration and deceleration).
9) Take out the centrifuge tube, and it can be seen that from top to bottom, the centrifuge tube contains washing solution, dead cell floaters, isolation solution, and pellet of mouse hepatic parenchymal cells (Figure 5). Discard the supernatant and retain the bottom layer of sediment, which consists of the live hepatocytes.

4. Hepatocytes Culture

1) Perform trypan blue staining on purified live hepatocytes, observe cell viability directly under a inverted microscope after staining, and then count with a cell counter.
2) According to the cell viability and the number of cells, inoculate at a viable cell density of 1.5×10^5 cells/cm^2.
3) Aspirate the Adhesion Culture Medium For Mouse Hepatic Parenchymal Cells into the coated culture vessel. Pipette the corresponding volume of hepatocytes suspension according to the calculated inoculation density, evenly drop it into the culture medium. Gently shake the cell suspension and then pipette it up and down to evenly distribute the cells (if the seeding density is uneven, it can easily cause cell aggregation in the middle of the vessel, resulting in phenomena such as cell clustering and floating).
4) After 4-6 hours of cell growth, observe under a microscope, it can be seen that the cells are significantly large and round, with double nuclei. Replace the culture medium with Maintenance Medium For Mouse Hepatic Parenchymal Cells.
5) After 24 hours of cell culture, cells begin to differentiate and gradually lose the characteristics of hepatocytes, with increasingly irregular morphology.

6) Change the medium every 2-3 days and the culture process can be sustained for 1-2 weeks.

Troubleshooting

Table 2. Common problems, causes, and solutions for isolation of Mouse Hepatic Parenchymal Cells

Problem	Possible Cause	Solution
During the perfusion process, the kidneys, right iliac region, and abdomen were distended.	The infusion needle punctured the blood vessel, causing the infusion fluid to flow to other areas.	Gently lift the venous vessel when inserting the needle, ensuring that the needle does not descend. Keep the needle stationary during the fixation process.
During the perfusion process, fluid accumulated in the abdominal cavity, and the liver gradually shrinked from expansion.	Excessive pressure can cause the rupture of blood vessels and tissues.	Ensure the hepatic portal vein is completely cut open, and do not clamp the hepatic portal vein for too long during the perfusion process.
The needle fall off and caused the accumulation of fluid near blood vessels during the perfusion process.	The length of the needle entering the blood vessel was too short.	It is advisable to insert the needle above the bifurcation of the inferior vena cava.
During the perfusion process, no distention was observed in the liver, and large areas of blood-red residue still remained in the liver tissue after perfusion and lavage.	The needle punctured blood vessels, the perfusion flow rate was too low.	Use scissors to clamp the hepatic portal vein, gently infuse the perfusion solution, and observe whether the liver is completely distended. If only partial or no distention occurs, it indicates that the needle has not been completely inserted into the blood vessel. Suggest re-experimenting.
After perfusion, it was difficult to peel off the liver capsule, and no quicksand-like cells was observed after peeling. After gentle shaking, the tissue maintained its normal shape.	Inactivation of digestive enzymes, prolonged preheating time, high flow rate during the perfusion digestion process led to insufficient digestion.	Check the storage conditions of digestive enzymes and complete the experiment within a short period of time after thawing. The preheating time should be controlled at around half an hour. During manual perfusion, use a timer to control the perfusion rate at around 5 mL/min.
After successful perfusion, the cell viability was low, and no sediment appeared at the bottom of the tube after isolation with the isolation solution.	Inadequate/excessive digestion.	Add digestive enzymes according to the recommended concentration and control the infusion rate.
During staining observation, cell clusters and lumps were observed.	Inadequate digestion.	Preheat the perfusion solution before perfusion. Ensure that an adequate amount of perfusion solution is infused.

Continued

Problem	Possible Cause	Solution
After isolation with the isolation solution, most of the cells settled at the bottom while some cells remained suspended in the isolation solution.	Excessive amount of cells were loaded.	Adjust the density of the cell suspension. If there are too many viable cells, add an additional 5 mL of cell washing solution to resuspend the precipitate.
A large number of cells floated within 24 h after inoculation	Excessive inoculation density can lead to cell death	Prior to inoculation, assess cell viability via staining, perform cell counting, and optimize the seeding density.
Cells grow poorly after inoculation.	Improper preparation of the culture medium and faliure to change the medium within the specified time.	Operate according to Procedure 1, Step (1), and Procedure 4, Step (4).
After inoculation, the cells tend to aggregate in the center of the culture dish while relatively fewer cells were observed inthe surrounding areas.	Uneven distribute of cells	When seeding, evenly drip the cell suspension dropwise and do not directly inject the entire volumn into the culture medium. After gently shaking the vessel, use a pipette tip or pipette to gently mix cells by pipetting up and down. Before placing the cell culture dish in the incubator, observe and confirm with a microscope.

Anatomy Images for Reference

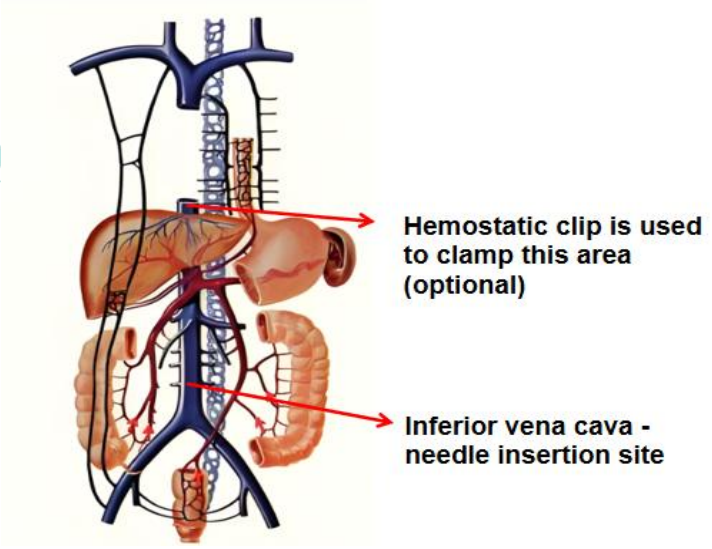

Figure 1. Portal vein system and perfusion needle site

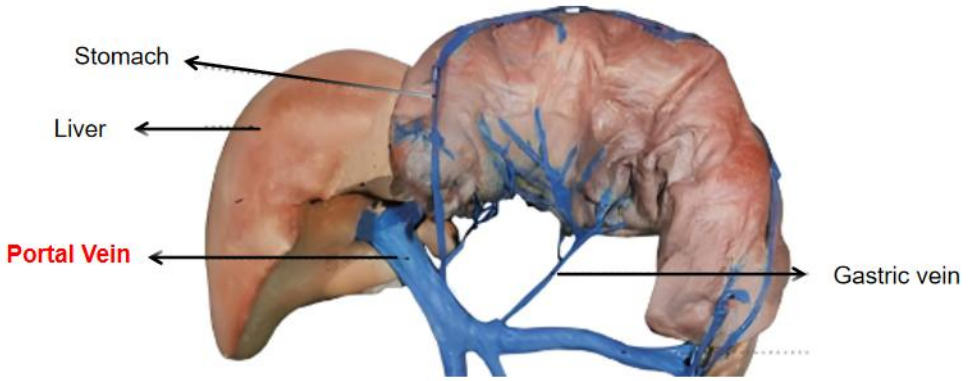

Figure 2. Schematic diagram of hepatic portal vein anatomy

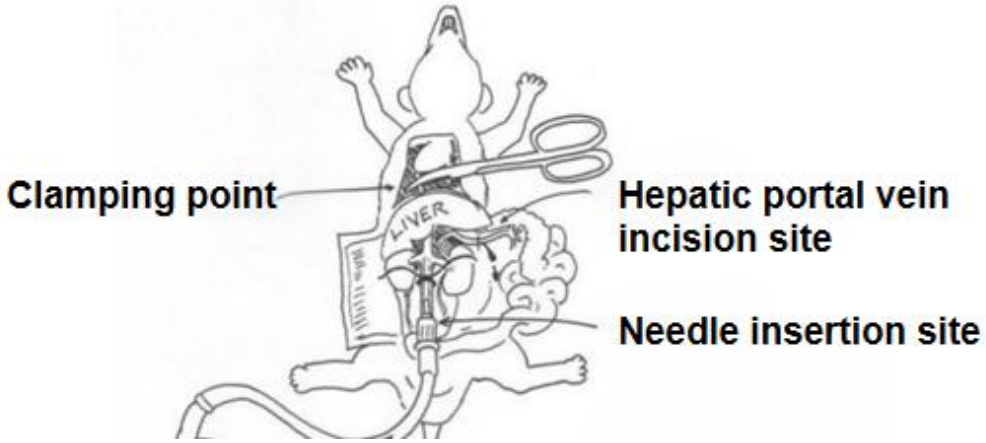

Figure 3. Overview diagram of perfusion process

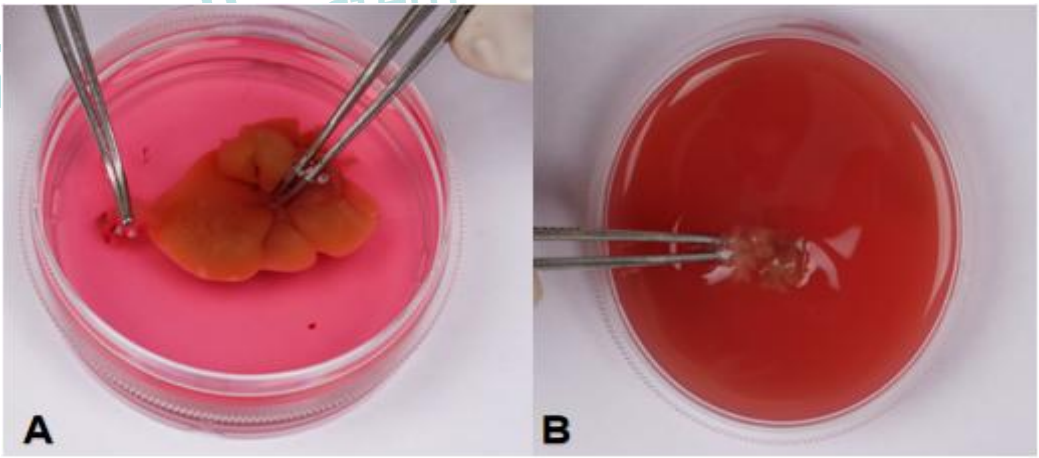

Figure 4. Treatment of the liver in vitro

A: Gently peel off the hepatic capsule. B: Gently shake the tissue to allow the cells to be released, leaving behind only a small portion of the residual tissue

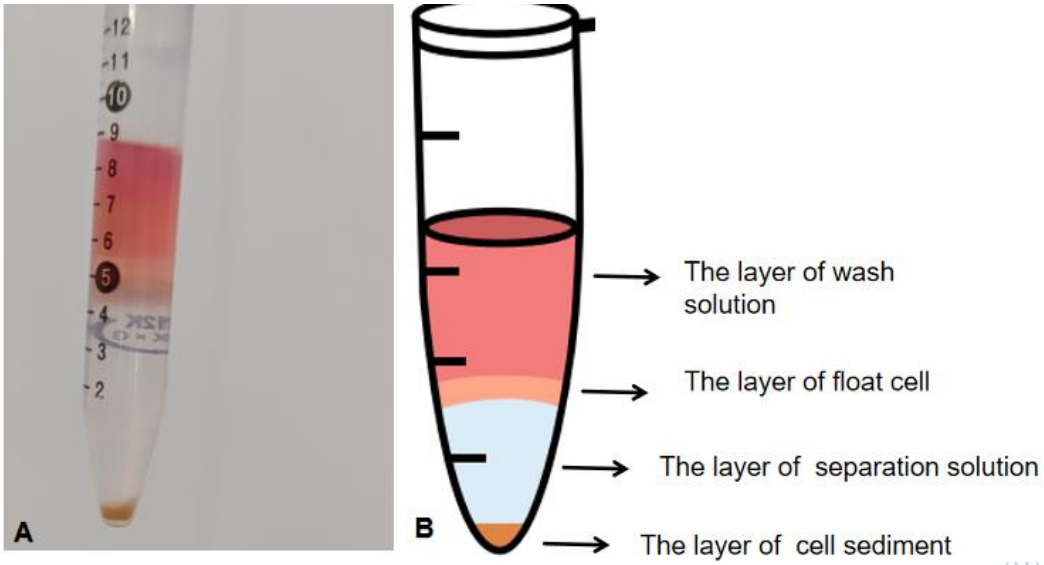

Figure 5. Stratification of cell suspension after centrifugation treated with isolation solution

A: Actual photo. B: Schematic diagram.