Rat Kupffer Cell Isolation and Culture Kit

Cat.No.: P-CA-613

Size: 3Tests/10Tests

Background

This kit is developed specifically for extracting primary rat kupffer cells. After low-temperature digestion with mixed enzymes and density gradient separation, the cells were purified using differential adhesion method to obtain rat kupffer cells. Laboratory validation showed that 1 × 10^6 target cells could be isolated in each experiment (1 Test). Immunofluorescence identification showed that the cell purity (CD68 positive rate) was > 90%.

Scope of Application

This product is suitable for extracting kupffer cells from different strains of mice such as Wistar, SD aged 14-84 days. 1-2 mice aged 14-28 days or 1 rat aged 35-84 days were used for each experiment to obtain intact liver tissue. After digestion, separation, and purification for 48 hours, kupffer cells with a quantity of >1 × 10^6 cells were obtained. Kupffer cells belong to terminally differentiated cells and do not proliferate. After separation, the cells can be cultured for about 1 week.

Note: The specific cell count may vary depending on the size and quantity of the tissue obtained from the sample.

Components of Kit

The components of this kit is shown in the table below

Table.1.Composition and corresponding information of rat kuppfer isolation kit

Name	Size	Appearance	Storage and Expiration Date
Specialized Cleaning Solution For Rat Kupffer Cells	3Tests (250 mL) 10Tests (500 mL×2)	Faint Yellow Clear Liquid	2-8°C, 1 year
Dilution Of Digestive Enzyme Of Rat Kupffer Cells	3Tests (60 mL) 10Tests (200 mL)	Red Clear Liquid	2-8°C, 1 year
Specific Digestive Enzyme For Rat Kupffer Cells	3Tests (6 mL) 10Tests (20 mL)	Brown Clear Liquid	-5~-20°C, 1 year
Special Separation Solution A For Rat Kupffer Cells	3Tests (20 mL) 10Tests (75 mL)	Faint Yellow Clear Liquid	2-8°C, 1 year
Special Separation Solution B For Rat Kupffer Cells	3Tests (20 mL) 10Tests (75 mL)	Faint Yellow Clear Liquid	2-8°C, 1 year
Special Separation Solution C For Rat Kupffer Cells	3Tests (10 mL) 10Tests (30 mL)	Yellow Clear Liquid	2-8°C, 1 year
Basic Culture Medium For Rat Kupffer Cells	3Tests (100 mL) 10Tests (300 mL)	Red Clear Liquid	2-8°C, 1 year
Supplements For Rat Kupffer Cells	3Tests (10 mL) 10Tests (30 mL)	Yellow Clear Liquid	-5~-20°C, 1 year
70 μm Cell Filter	3Tests (3 pcs) 10Tests (10 pcs)	N/A	Room temperature, 3 years

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Name	Size	Appearance	Storage and Expiration Date
100 μm Cell Filter	3Tests (3 pcs) 10Tests (10 pcs)	N/A	Room temperature, 3 years

Note: Please store each component according to the temperature indicated on the label on the reagent tube. After thawing, the digestive fluid should be stored at 4℃ for 30 days. It is recommended to divide the digestive fluid according to the instructions after the first use of the reagent kit, freeze it in a -20℃ refrigerator, and thaw it again before use to avoid repeated freezing and thawing.

Notes

1. This product is only used for scientific research or further research, not for diagnosis and treatment.
2. The experiment requires the preparation of dissection plates, ice plate, 0.25% trypsin digestion solution, and surgical instruments (including 2 ophthalmic scissors, 2 surgical straight forceps, and 1 surgical curved forceps); tissue processing dishes (glass culture dishes, 6 cm/10 cm are acceptable), 6 cm culture dishes, and several 15 mL/50 mL centrifuge tubes.
3. Before the formal experiment, it is recommended to use 1-2 normal rats for simulated anatomy to familiarize the operation process and improve the speed of tissue separation.
4. During the entire separation process, it is recommended to place the culture dish containing the tissue on an ice plate (2-8°C) to maintain low temperature, but be careful not to freeze the tissue and liquid.
5. The culture medium contains nutrients necessary for microbial growth. Please open it in an ultra-clean workbench, divide it according to the required amount, and seal the bottle mouth with a sealing film for immediate use to avoid contamination.
6. All separation solutions in this kit may experience the precipitation of crystal particles at the bottle mouth and turbidity of the solution during storage, which is a normal phenomenon and can be used with confidence.

Operational Procedures

1. Pre-experiment Preparation

(1) Reagent Thawing and Rewarming: **Specific Digestive Enzyme For Rat Kuppfer Cells**,**Supplements For Rat Kupffer Cells**: Thaw at 4°C and equilibrate to room temperature. **Specialized Washing Solution For Rat Kupffer Cells**, **Diluent of Digestive Enzyme For Rat Kupffer Cells**, **Special separation Solution A For Rat Kupffer Cells**, **Special Separation Solution B For Rat Kupffer Cells**, **Special Separation Solution C For Rat Kupffer Cells**, Basic Culture Medium For Rat Kupffer Cells: Equilibrate to room temperature.

(2) Preparation of **complete culture medium**: Add 5 mL of Supplements For Rat Kupffer Cells into 50 mL of Basic Culture Medium For Rat Kupffer Cells, mix thoroughly.

(3) Preparation of **digestive working solution for Rat Kupffer cells**: Add 2 mL **Specific Digestive Enzyme For Rat Kupffer Cells** into 18 mL Diluent of **Digestive Enzyme For Rat Kupffer Cells**,mix thoroughly.

(4) Perform euthanasia via pentobarbital sodium overdose injection or cervical dislocation, then immerse the animal in 75% medical-grade ethanol for 5 minutes for disinfection. After sterilization, transfer the animal to a clean bench for subsequent procedures.

2. Dissection Protocol

(1) Preparation: Place two clean culture plates in the clean workbench, and place ophthalmic scissors 1, straight forceps 1, ophthalmic scissors 2, straight forceps 2, ophthalmic scissors 3, and curved forceps 1 from left to right above the culture plates. Pay attention to placing ophthalmic scissors and forceps in pairs. The distal third of the instruments should extend beyond the rack to avoid contamination. After each use, return tools to their original positions and make sure they don't touch each other to prevent cross-contact.

(2) Rat fixation: Secure the rat in a supine position within the clean workbench using needles for stabilization during tissue harvesting.

(3) Sampling procedure: Use ophthalmic scissors 1 to longitudinally cut the open chest skin along the midline of the abdomen, lift the skin along the opening with straight forceps 1, and use ophthalmic scissors 1 to horizontally cut the skin in the direction of the limbs, bluntly peel off the skin and subcutaneous tissue on both sides, expose the superficial muscle layer of the abdominal wall, and use straight forceps 1 to pull the skin to both sides. Exchange for another set of ophthalmic scissors 2 and straight forceps 2, cut open the peritoneum, fully expose the liver tissue, remove the connective tissue and blood vessels around the liver with ophthalmic scissors 2, take out the complete liver tissue, place the tissue in a glass culture dish, and pre add **Specialized Washing Solution For Rat Kupffer cells** to the dish to immerse the tissue.

3. Tissue Processing and Digestion

(1) Tissue Washing: Using a new set of ophthalmic scissors 3, curved forceps 1, bend forceps 1 to clamp the junction of the liver lobes and gently shake the liver tissue. At this time, blood stains flow out. discard the liquid in the dish and add new wash solution until the tissue is completely immersed. Rinse the tissue 2-3 times and use ophthalmic scissors 3 and curved forceps 1 to clean the blood stains, bile ducts, and blood vessels around the liver to obtain pure tissue. Transfer the tissue to a new glass culture dish, Add a little of **Specialized Washing Solution For Rat Kupffer cells** until it just covers the tissue.

(2) Tissue Processing：Tilt the dish about 45° to allow the liver tissue to gather at the bottom of the dish(which can be gently scraped off with curved forceps 1). Quickly cut the tissue into 1 mm^3 pieces using ophthalmic scissors 3, until the tissue is sugar like (as shown in Figure 1) and there are no large pieces of tissue. Use a Pasteur pipette or a pipette with a wide mouthed tip to transfer the tissue fluid and transfer it to a 50 mL centrifuge tube. Add 5 mL **Specialized Washing Solution For Rat Kupffer Cells** to clean the liver tissue culture dish and transfer it to the same centrifuge tube. Centrifuge at 300 g for 1 min, discard the supernatant, and retain the tissue precipitate.

(3) Tissue Digestion: Add 20 mL of **Digestive Working Solution for Rat Kupffer Cells** to the 50 mL centrifuge tube tissue sediment, blow and mix well, tighten the bottle mouth, wrap it with a sealing film to ensure no leakage, tilt it to allow tissue fragments to fully contact the digestion solution at 4°C for 16 hours of digestion (digestion time should not exceed 16 hours, as excessive digestion will have a significant impact on cell activity).

(4) After digestion, remove the centrifuge tube and transfer it to a 37°C water bath shaker at 150 rpm for 15 minutes of digestion..

(5) In the ultra-clean workbench, place a 70 μm Cell filter and 100 μm Cell Filter on a new 50 mL centrifuge tube. Pre-wash the filter with1-2 mL Specialized Washing Solution For Rat Kupffer Cells.

(6) Cell Filtration: Remove the centrifuge tube and open it in the ultra-clean workbench. Blow the tissue suspension with a 5 mL pipette tip at least 30 times. Add 5 mL of **Specialized Washing Solution For Rat Kupffer Cells** to dilute. Take the cell suspension at this time and filter it through a 100 μm cell filter and a 70 μm cell filter in sequence to collect the filtrate.

4. Cell isolation, purification, and cultivation

(1) Centrifuge 400 g of the cell filtrate obtained in the above steps for 10 minutes, discard the supernatant, and retain the precipitate.
(2) Resuspend the precipitate using 6 mL of **Special Separation Solution A For Rat Kupffer Cells** ,centrifuge 900 g for 15 minutes.
(3) After centrifugation, take out the centrifuge tube, discard the supernatant (which contains hepatic stellate cells, a small amount of hepatic sinusoidal endothelial cells, Kupffer cells, and parenchymal cells), and retain the pellet. Resuspend the pellet using 6 mL of **Special Separation Solution B For Rat Kupffer Cells**. Centrifuge the tube at 900 g for 15 minutes.
(4) Take a new 15 mL centrifuge tube and add 3 mL of **Special Separation Solution C For Rat Kupffer Cells** for later use.
(5) After centrifugation, take out the centrifuge tube and discard the supernatant (The supernatant consists of cell debris and a small amount of cells), while retaining the precipitate. Resuspend and precipitate using 5 mL **complete culture medium.**
(6) Use a 1 mL pipette to collect the cell suspension from the 50 mL centrifuge tube, tilt the tube with the pipette tip against the wall of the tube, and gently blow out the cell suspension along the tube wall to slowly flow into the liquid surface above **Special Separation Solution C For Rat Kupffer Cells** (as shown in Figure 2), form two layers of liquid (as shown in Figure 3).
Note: If it is not ensure that form two layers of liquid, a 200 μL pipette can be used to aspirate the resuspended cell suspension and slowly blow out it along the tube wall for about 5-6 shots according to the above operation. After obvious liquid level stratification is seen, use a 1 mL pipette to slowly add the remaining suspension. The liquid level stratification can be observed under light, and a clear boundary line can be seen between the two liquid.
(7) After stratification,centrifuge tube at 900 g for 15 minutes (set the acceleration to 1 and the decelerate to 0); After centrifugation, the liquid can be seen to be divided into 5 layers, from top to bottom:the top layer is approximately 1 mm thick Cellular debris layer, the second layer is approximately 5 mL complete culture medium, the third one is approximately 2-3 mm thick, liver sinusoidal endothelial and kupffer mixed cells (hereinafter referred to as the white membrane layer), the fouth is approximately 3 mL Separation solution C, and the bottom layer is approximately 2-5 mm thick red blood cell precipitation (as shown in Figure 4).
(8) Discard the top layer of cells and use a 1 mL pipette tip above the white membrane layer. Carefully remove the cells from the third layer of white membrane layer into a new sterile 15 mL centrifuge tube, add 5 mL of **Specialized Washing Solution For Rat Kupffer Cells** to resuspend the cell suspension, centrifuge at 400 g for 5 min, discard the supernatant, and retain the precipitate.
(9) Resuspend 5 mL of **Complete Culture Medium for Rat Kupffer Cells** and seeding it into a new 6 cm culture dish.
(10) Incubate in a 5% CO_2 incubator at 37°C for 25 minutes, then transfer the supernatant to a new culture dish. Add 5 mL of fresh **Complete Culture Medium for Rat Kupffer Cells** to the original culture dish and continue culturing at 37°C for 25 minutes.
(11) Transfer the transferred supernatant again to a new dish, add fresh complete culture medium to the original dish, and repeat this transfer process 1-2 times.
(12) After differential adhesion, most of the kupffer cells exist in the dish seeded in step 8, while the rest of the dishes only have a small or partial amount of kupffer cells, which can be selected according to the specific cell purity and cell quantity.If the number of adherent cells is small, they can be cultured directly. The detailed process is shown in item 7 of Table 2.
(13) After 48 hours of cultivation, collect the target cells and rapidly digest them with 0.25% trypsin for about 1 minute. Discard the first digested cells, which may contain loosely adherent liver sinusoidal endothelial cells. Gently rinse the bottom of the dish with a **Specialized Washing**

Solution For Rat Kupffer Cells, discard the solution, add fresh complete culture medium, and change the medium every 2-3 days thereafter.

(14) Cell digestion: It is generally not recommended to perform digestion and passage operations on kupffer cells. If it is necessary to transfer the cells in the original dish, please follow the following steps.

a. Discard the original supernatant, add 2-3 mL of PBS to rinse the cells, discard PBS, add 1 mL of 0.25% trypsin digestion solution, gently shake until all cells are infiltrated into the flask, and digest in a 37°C incubator for 2-5 minutes.

b. After most of the cells have shrunk and become round, add 3-5 mL of complete culture medium to terminate digestion. Gently blow and disperse the cells, according to the experimental requirements, lay the cells in proportion, conduct the experiment, and place them in a cell culture incubator at 37°C, 5% CO_2, and saturated humidity for static cultivation

Troubleshooting

Table.2.Common problems, causes, and solutions of kupffer cells Separation in rats

Problem	Possible Cause	Solution
A large amount of tissue block are deposited at the bottom of the tube (procedure3-step (4))	Insufficient tissue digestion (excessive amount of tissue sampling, improper placement of centrifuge tubes during digestion, etc.)	Cut the tissue into a sugar like shape. The centrifuge tube is laid horizontally.Take an appropriate amount of the tissue, the volume of the tissue sediment should not exceed one third of the volume of the digestion solution.
After rewarming digestion, the tissue cannot be completely blown away, and there are still block like tissues (procedure3-step (6))	Inadequate digestion	Confirm the storage conditions of digestive enzymes and whether completely cut during tissue processing; Grinding tissue can be considered without influence cells active.
No obvious 5 layers were observed after centrifugation of the Separation system (procedure4-step (6))	Overdigestion leads to cell death	Follow the instructions in Step 5 of procedure 4 of the procedure. During centrifugation, use the slowest speed for both ascending and descending.
No cell white membrane layer was observed in the target layer after centrifugation of the separation system. (procedure4-step (6))	Overdigestion leads to cell death	Control the low-temperature digestion time to not exceeding 16 hours.
Low cell quantity	The volum of tissue obtain is few, Inadequate differential attachment time	Strictly follow the instructions for rat age and number (obtaining too little liver tissue may increase the number of extracted rats); Check if the cells are retained in the dish of the first two steps of differential attachment, and adjust the differential attachment time based on the results.
Low cell purity, mixed with other cells	The cell fluid contains liver sinusoidal endothelial cells	Purification of liver sinusoidal endothelial cells using differential digestion method (kupffer cells have strong adhesion and are difficult to digest, and can be digested with trypsin for 1-2 minutes to transfer the digested liver sinusoidal endothelial cells).

Continued

Problem	Possible Cause	Solution
After differential adhesion, the number of cells is low and the purity is low.	During the differential adhesion process, some liver sinusoidal endothelial cells adhere to the wall.	After seeding the cell pellet, do not change the medium, and continue to culture (Kupffer cells have low nutritional requirements, and during this process, miscellaneous cells will gradually die) until the culture medium in the dish turns yellow.

Anatomy Images for Reference

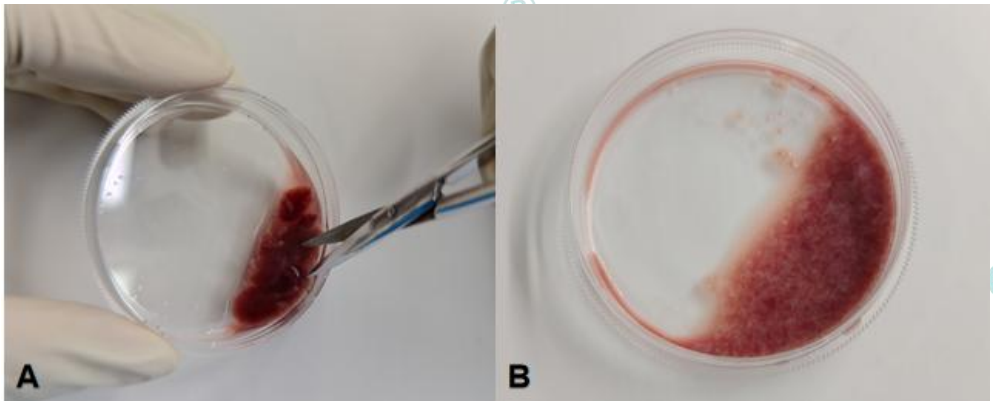


Figure 1. Treatment of liver tissue in vitro
A: Demonstration of liver tissue cutting operation; B: liver after cutting into pieces

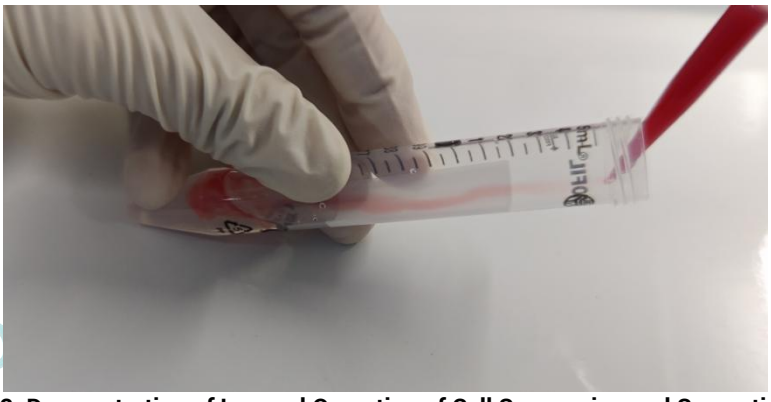


Figure 2. Demonstration of Layered Operation of Cell Suspension and Separation solution

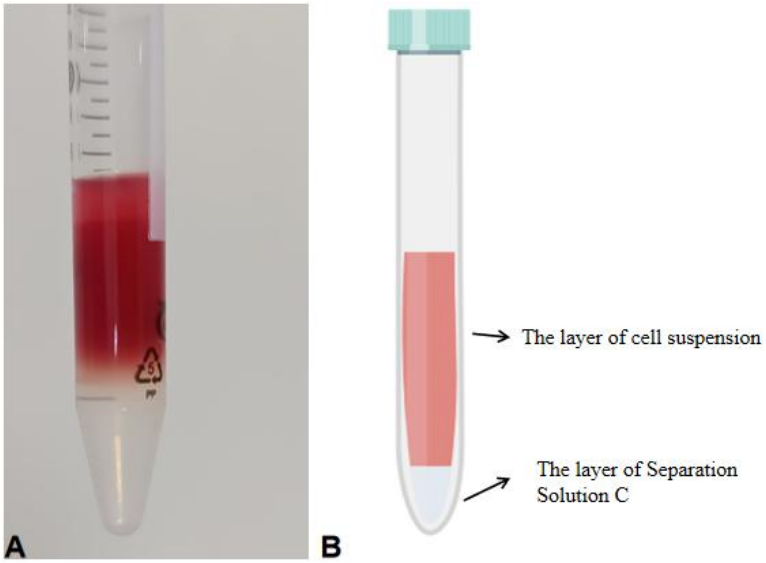
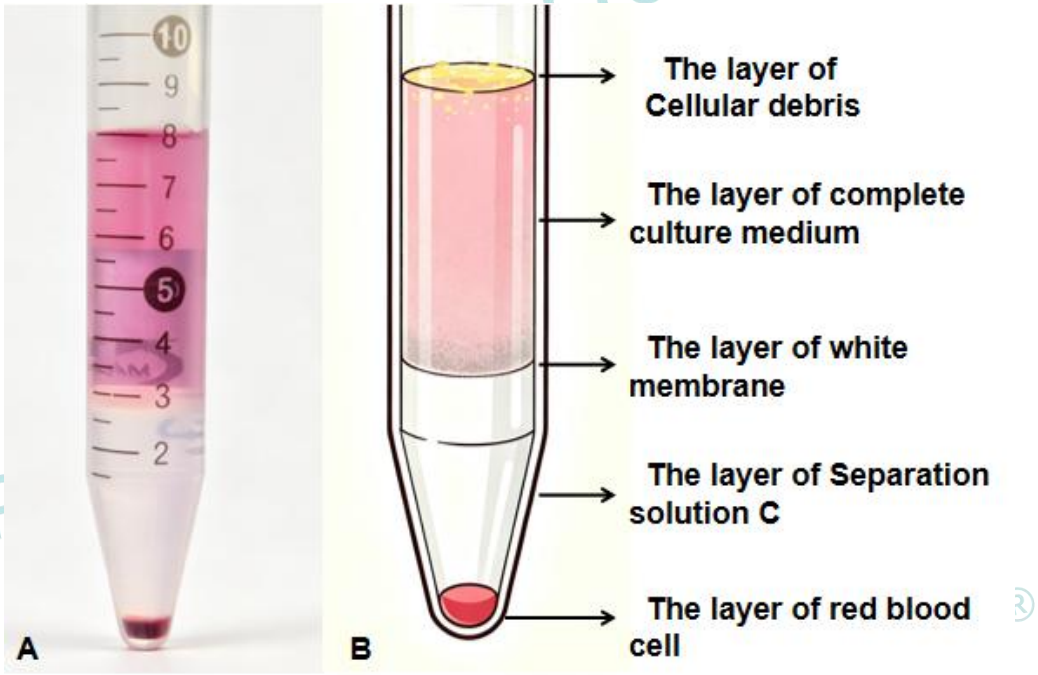


Figure 3. Reference diagram for liquid level stratification
A: Real photos of cell suspension and Separation solution C after stratification; B: Schematic diagram of cell suspension and Separation solution C after stratification

Figure 4. Reference image of cell suspension after centrifugation with Separation solution



A: Cell suspension was centrifuged with Separation solution and layered in real photos;
B: Schematic diagram of layering of cell suspension after centrifugation with Separation solution
(For reference only, the thickness of the cell layer and the color depth of the liquid may vary depending on experimental conditions)