# Rat Hepatic Stellate Cell Isolation and Culture Kit

**Cat.No. : P-CA-612**

**Size：3Tests / 10Tests**

## Background

The Rat Hepatic Stellate Cell Isolation and Culture Kit is specially developed for extracting primary Rat Hepatic Stellate Cells. As validated, standard operation using this kit enables the acquisition of one flask of cells (T-25 culture flask) per 1 Test,with a cell count exceeding 1 × $10^6$ cells. The cells can be passaged at a ratio of 1:2 for about 3 times. Through immunofluorescence identification, the cell purity (α - SMA positive rate) has been confirmed to exceed 90%.

## Scope of Application

This product is suitable for extracting Hepatic Stellate Cells from rats aged 1-2 days of various strains,such as Wistar and SD..Through processes of tissue isolation, digestion, and 48-hour planting purification, a yield of >1×$10^6$ cells can be obtained.

**Note: Extracting complete liver tissue from 5 rats can obtain cells in a T25 culture bottle. The specific number of rats required may vary depending on the amount of liver tissue obtained.**

## Kit Components

The components of this kit is shown in the table below

Table.1.Composition and corresponding information of Rat Hepatic Stellate Cell Isolation and Culture Kit

| Name | Size | Appearance | Storage and Expiration Date |
|---|---|---|---|
| Specialized Washing Solution For Rat Hepatic Stellate Cells | 3Tests (250 mL)<br>10Tests (500 mL) | Faint yellow Clear Liquid | 2-8°C,1 year |
| Diluent of Digestive Enzyme For Rat Hepatic Stellate Cells | 3Tests (15 mL)<br>10Tests (50 mL) | Colorless Clear Liquid | 2-8°C,1 year |
| Specific Digestive Enzyme For Rat Hepatic Stellate Cells | 3Tests (1.5 mL)<br>10Tests (5 mL) | Faint yellow Clear Liquid | -5~-20°C,1 year |
| Specific Separation Solution A For Rat Hepatic Stellate Cells | 3Tests (15 mL)<br>10Tests (50 mL) | Colorless Clear Liquid | 2-8°C, shading light, 1 year |
| Specific Separation Solution B For Rat Hepatic Stellate Cells | 3Tests (15 mL)<br>10Tests (50 mL) | Colorless Clear Liquid | 2-8°C, shading light, 1 year |
| Basic Culture Medium For Rat Hepatic Stellate Cells | 3Tests (100 mL)<br>10Tests (300 mL) | Red Clear Liquid | 2-8°C, 1 year |
| Supplement For Rat Hepatic Stellate Cells | 3Tests (10 mL)<br>10Tests (30 mL) | Brown Clear Liquid | -5~-20°C, 1 year |
| 100 μm Cell Filter | 3Tests (3 pcs)<br>10Tests (10 pcs) | Green | Room temperature, 3 years |
| 70 μm Cell Filter | 3Tests(3 pcs)<br>10Tests(10 pcs) | Orange | Room temperature, 3 years |

**Note: All components should be stored according to the temperature indicated on the labels of the reagent tubes. The reagentsstored at -5~-20℃ (such as Specialized Digestive Solution, Supplement For Rat Hepatic Stellate Cells ) can be preserved at 4℃ for 30 days after thawing. For long-term storage, aliquot them into single-use portions and store at**

**-20℃. Thaw again at 4℃ before use to avoid repeated freeze-thaw cycles.**

## Notes

1. Prior to formal experiments, it is recommended to conduct anatomical simulation training using 1-2 normal rats to familiarize operators with procedural workflows and improve tissue dissociation efficiency.
2. 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 due to low temperature, so as not to damage the tissue and affect the extraction efficiency.
3. Reagent preparation or dispensing must strictly adhere to aseptic technique protocols. After dispensing, seal the containers immediately with a sealing film, use them promptly to avoid repeated freeze-thaw cycles orcontamination.

## Operational Procedures

### 1. Pre-experiment Preparation

(1) Self-supplied Reagents and Consumables: two Eppendorf (EP) tube racks, Phosphate-Buffered Saline (PBS), Ice plate, surgical instruments (including at least 3 pairs of ophthalmic scissors, 2 pairs of straight tweezers, 2 pairs of bent tweezers), 6 cm/10 cm culture dish, T25 culture flask, dissecting plate (can be replaced by foam plate), several syringe needles, several 15 mL/50 mL centrifuge tubes, 0.25% trypsin digestion solution (for cell digestion and passage). If you need to expand cell culture, please prepare complete medium and trypsin in advance.

(2) Reagent Thawing and Rewarming:

① **Specific Digestive Enzyme For Rat Hepatic Stellate Cells**, **Supplement For Rat Hepatic Stellate Cells**: Thaw at 4°C and equilibrate to room temperature.

② **Specialized Washing Solution For Rat Hepatic Stellate Cells**, **Diluent of Digestive Enzyme For Rat Hepatic Stellate Cells, Specific Separation Solution A For Rat Hepatic Stellate Cells, Specific Separation Solution B For Rat Hepatic Stellate Cells**, **Basic Culture Medium For Rat Hepatic Stellate Cells** (Separation Solution A and B need to avoid light): Equilibrate to room temperature.

③ **Preparation of Complete Culture Medium For Rat Hepatic Stellate Cells**: Add 5 mL of **Supplement For Rat Hepatic Stellate Cells** into 50 mL of **Basic Culture Medium For Rat Hepatic Stellate Cells**, mix thoroughly.

④ Preparation of **Digestion Working Solution For Rat Hepatic Stellate Cells**: Take 500 μL of **Specific Digestive Enzyme For Rat Hepatic Stellate Cells,** add 5 mL of **Diluent of Digestive Enzyme For Rat Hepatic Stellate Cells,** mix well and set aside for later use.

**Note: Storage conditions for complete Culture Medium For Rat Hepatic Stellate cells: 2-8℃, time of validity: 3 months; The storage conditions for the rat hepatic stellate cell digestion solution after preparation : 2-8℃ and the time of validity is 1 month. The remaining additives need to be packaged in proportion and stored in a -5 ~ -20℃ refrigerator to avoid repeated freezing and thawing.**

### 2. Dissection Protocol

(1) Animal disinfection and euthanization: Use the gradual cooling method to euthanize mice, and

immerse them in 75% medical alcohol for 5 minutes for disinfection. After disinfection is completed, transfer the animals to a clean bench for subsequent operations.

(2) Dissection and Tissue Harvesting Steps:

① Preparation： Arrange sterilized scissors and instruments in the order of use from left to right above the disinfected sterilized EP tube rack in the ultra clean beach: ophthalmic scissors 1 and straight tweezers 1, ophthalmic scissors 2 and straight tweezers 2, curved tweezers 1, ophthalmic scissors 3 and curved tweezers 2.

**Note: 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 bench using needles for stabilization during tissue harvesting.

③ Tissue Sampling:

a. Gently lift the abdominal skin of the rat with straight tweezers 1 on the left hand, and use the tip of ophthalmic scissors 1 on the right hand to make a small incision under the skin. Be careful not to cut through the peritoneum. Use straight tweezers 1 to tear open the skin from the opening of the skin and pull the abdominal skin to the neck of the rat, exposing the abdominal cavity.

**Note: Cut the skin to expose the entire abdominal cavity, and be careful to tear the skin away from the anatomical area to prevent contamination.**

b. Exchange for ophthalmic scissors 2 and straight tweezers 2, use straight tweezers 2 to pull up the peritoneum, and ophthalmic scissors 2 to cut open the peritoneum and pull it to both sides, so that the liver tissue can be completely exposed. Open the abdominal cavity, cut open the blood vessels and membranes connected to the abdominal cavity along the lower part of the chest cavity, use curved tweezers 1 to pick out the liver tissue, completely remove the liver tissue, and place it in a culture dish containing 5-10 mL of **Specialized Washing Solution For Rat Hepatic Stellate Cells**. Place the entire culture dish on an ice plate to maintain a low temperature environment.

**Note: During the entire sampling process, only the first set of scissors and tweezers can come into contact with the external skin of the rat. Other instruments are strictly prohibited from touching the external skin and hair. If touched, sterile instruments must be replaced to prevent contamination. If the operation time is long, it is necessary to observe and gently shake the culture dish from time to time to prevent the tissue and washing solution from freezing due to prolonged contact with the ice plate which may damage the tissue and affect the extraction effect.**

## 3. Tissue Processing and Digestion

(1) Tissue Processing

① Place ophthalmic scissors 3 and curved forceps 2 above the EP tube rackr in the clean bench, with the front end suspended.

② Using this new set of scissors, operate on liver tissue: rinse the liver tissue in the dish with **Specialized Washing Solution For Rat Hepatic Stellate Cells** 1-2 times using curved tweezers 2, clean the blood around the liver, and remove bile ducts and blood vessels using ophthalmic scissors 3 to obtain pure tissue.

③ Transfer the obtained pure tissue to a new culture dish, lift the dish at an angle of about 45 º, use tweezers to move the tissue to the bottom, add a small amount of **Specialized Washing Solution For Rat Hepatic Stellate Cells** until it just covers the tissue, quickly cut the tissue into 1 mm3 pieces using an ophthalmic scissors (the flesh can be observed as sugar like), add 5 mL of

**Specialized Washing Solution For Rat Hepatic Stellate Cells** to blow away the tissue pieces, transfer the tissue pieces to a 15 mL centrifuge tube, resuspend, centrifuge at 1200 rpm for 1 min, discard the supernatant, and retain the tissue block precipitate.

(2) Organizational digestion: Add 5 mL of **Specific Digestive Enzyme For Rat Hepatic Stellate Cells** into the centrifuge tube, gently blow and mix, wrap the centrifuge tube tightly with sealing film, place the centrifuge tube in a 37°C water bath shaker, adjust the speed to 150 rpm, and digest for 30-40 minutes.

## 4. Cell Purification

(1) Place a **70 μm Cell Filte**r and **100 μm Cell Filter** on a new 50 mL centrifuge tube. Pre-wash the filter with1-2 mL washing solution for later use.

(2) After the digestion is completed, take out the centrifuge tube and repeatedly blow the tissue inside the tube with a 5 mL pipette for more than 30 times until there are no obvious large pieces of tissue observed by the naked eye. Add 5 mL of **Specialized Washing Solution For Rat Hepatic Stellate Cells** to the centrifuge tube, gently resuspend and dilute the digestion solution in the tube, then draw the tissue digestion suspension, filter it through a **100 μm Cell Filter**, draw another 50 mL of filtrate from the centrifuge tube, filter it through a **70 μm Cell Filter**, and after filtration is complete, slowly add 3-5 mL of washing solution to the top of the filter with a clean pipette tip to collect the tissue digestion suspension on the filter and the cell filtrate in the collection tube.

**Note: Filtration criteria: The suspension is easy to pass through the filte without a large amount of gelatinous substances. If the suspension filtration is slow or unable to be filtered during this step, it may be due to the tight fit between the cell filte and the centrifuge tube mouth. At this time, you can try tilting the cell filte slightly against the tube rim to improve this phenomenon.**

(3) Place a 50 mL centrifuge tube containing filtered tissue suspension, centrifuge at 400 g for 10 minutes, discard the supernatant, add 4 mL of **Specific Separation Solution B For Rat Hepatic Stellate Cells** to the 50 mL centrifuge tube, resuspend the precipitate, transfer the cell suspension to a new 15 mL centrifuge tube, and slowly inject 4 mL of **Specific Separation Solution A For Rat Hepatic Stellate Cells** along the tube wall above the cell suspension **(this is a key step, the operation should be as gentle as possible during the process to ensure that the solution flows down the tube wall after being blow out)**, so that there is a clear liquid level stratification between the two. Finally, slowly add 2 mL of **Complete Culture Medium For Rat Hepatic Stellate Cells** along the tube wall to form a complete culture medium with the liquid below. Liquid level stratification, centrifuge at 1400 g for 30 minutes (set the acceleration to 1 and the decelerate to 0).

(4) After centrifugation, the liquid inside the tube appears as four layers, from top to bottom: medium layer, white membrane layer, separation solution A layer, and separation solution B. The white membrane layer at the junction of rat hepatic stellate cell specific separation solution A and culture medium (the second layer from top to bottom) is aspirated, and 1-2 mL of liquid above and below the white membrane layer is aspirated with a pipette tip and transferred to a new 15mL centrifuge tube. 10 mL of **Specialized Washing Solution For Rat Hepatic Stellate Cells** is added to resuspend the white membrane layer suspension, centrifuge at 400 g for 30 minutes. Discard the supernatant and retain the precipitate.

(5) Resuspend the precipitate in 5 mL of **Complete Culture Medium For Rat Hepatic Stellate Cells**, aspirate the cell suspension and seed it into a 6 cm dish. Incubate in a 37°C, 5% $CO_2$ incubator for 40 minutes.

(6) Remove the culture dish, transfer the supernatant of culture medium from the dish to a new one, and incubate in a 37°C, 5% $CO_2$ incubator for 40 minutes. Add fresh culture medium to the original dish. Repeat this step twice. The dish seeded for the first time may contain other miscellaneous cells, such as kupffer cells and liver sinusoidal endothelial cells. The dishes of later seeded contain higher purity hepatic stellate cells.

## 5. Cell Culture and Subculture

(1) Medium replacement: Transfer the cell culture medium (containing cells) obtained in the previous step to a new T25 flask, incubate at 37 °C for 18 hours, and see that most of the cells have adhered. Discard the supernatant and add fresh culture medium. Afterwards,replace the medium every 2-3 days.

(2) Morphological observation: The newly isolated cells appeared circular, with most of cells became adherent after 12 hours. After 24 hours, the cells appeared circular or spindle shaped, and after 48 hours, the volume of the adherent cells increased and began to expand, showing a spindle or star shape. A few cells extended pseudopodia. After 5-7 days of cultivation, the number of cells significantly increased, appearing spindle or star shaped, extending pseudopodia, and the cells began to activate.

(3) Cell passaging protocol: Passage should be initiated when cells reach 80-90% confluency. Aspirate the old medium and rinse cells with 2-3 mL PBS to remove residual serum. Add 1 mL of 0.25% trypsin solution to the flask. Tilt the flask gently to ensure even coverage of the cell monolayer. Aspirate excess trypsin, leaving a thin layer to avoid over-digestion. Place the flask in a 37°C incubator for 1-3 minutes. Monitor under an inverted microscope until >80% of cells round up and detach. Add 3-5 mL **Complete Medium For Rat Hepatic Stellate Cells** to neutralize trypsin activity. Pipette gently to resuspend cells into a single-cell suspension. Transfer the cell suspension to new culture flasks at the desired split ratio. Ensure even distribution by swirling the flask. Incubate the flasks at 37°C in a humidified atmosphere with 5% $CO_2$ and saturated humidity.

## Troubleshooting

Table.2.Common problems, causes, and solutions of Rat Hepatic Stellate Cells

| Problem | Possible Cause | Solution |
|---|---|---|
| After digestion is complete, there is transparent adhesive bonding between tissues. | Insufficient digestion due to excessive sample size | Take an appropriate amount of tissue The volume of digestive fluid is greater than or equal to three times that of the tissue block. |
| After digestion, there are still some tissue blocks that cannot be blown away, and there are suspended flocculent and strip-shaped tissues in the separated solution B. | Insufficient tissue digestion | Check the tissue quantity and the degree of fragmentation.Extend digestion time appropriately. |
| After digestion, there are many connective tissues that cannot be disperse. | Excess tissue was not removed during the sampling process | Normal experiment, filter the remaining tissues with a filter. |

Continued

| Problem | Possible Cause | Solution |
|---|---|---|
| After separation, no white membrane layer was observed or the white membrane layer was not obvious. | There is no liquid level stratification formed between the separated solutions, the failure of the separated solution. | The separation solution should be stored away from light. Please remove it from a dark place when using it. Strictly follow the standard operation to Form liquid level stratification between the two |
| The final obtained cells have low purity. | Differential adhesion did not remove other cells. | Perform secondary differential adhesion, digest the cells, and refer to procedure4-step(6) for operation. |
| On the second day after differential adhesion, it was observed that there were fewer cells. | The differential attachment time is too long. | Suggest keeping all culture dishes after differential adhesion and observing which step of the culture dish the target cells are retained in |
| Poor cellular state. | The preparation of the culture medium was incorrect, the storage conditions were improper, and the medium was not changed timely. | Ensure the correct proportion of the complete medium, avoid repeated freezing and thawing, and promptly change the medium after purification and separation. |
| Slow cell proliferation | Inappropriate passage ratio and the Over-passaged | calculate based on vessel surface area to maintain proper cell seeding density.Limit cell passage to 2-3 times to prevent proliferation slowdown |