Mouse Liver Sinusoidal Endothelial Cells Isolation and Culture Kit

Cat.No. : P-CA-709

Size：3Tests/10Tests

Background

The Mouse liver Sinusoidal Endothelial Cells Isolation and Culture Kit is specifically developed for extracting primary mouse liver sinusoidal endothelial cells. After 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 When passaged at a 1:2 ratio, the cells can undergo 2-3 passages. Through immunofluorescence analysis, the cell purity (CD31/CD14-positive rate) has been confirmed to exceed 90%.

Scope of Application

This product is suitable for extracting liver sinusoidal endothelial cells from mice aged 14-84 days of various strains. Through processes of tissue isolation, digestion, and 48-hour planting purification a yield of $> 1 \times 10^6$ cells can be obtained.

Note: Sufficient cells for culturing in a T25 flask can be obtained from the complete liver tissue of either 1-2 mice aged 14-28 days or a single mouse aged 35-84 days The exact number of mice required may vary depending on the siaze and quntity of liver sinusoidal endothelial tissue harvested during the procedure.

Components of Kit

The components of this kit is shown in the table below

Table.1.Composition and corresponding information of mouse sinusoidal endothelial isolation kit

Name	Size	Appearance	Storage and Expiration Date
Specialized Washing Solution For Mouse Liver Sinusoidal Endothelial Cells	3Tests (250 mL) 10Test (500 mL×2)	Faint Yellow Transparent Liquid	2-8℃, 1 year
Diluent of Digestive Enzyme For Mouse Liver Sinusoidal Endothelial Cells	3Tests (15 mL) 10Tests (50 mL)	Colorless Transparent Liquid	2-8℃, 1 year
Specific Digestive Enzyme For Mouse Liver Sinusoidal Endothelial Cells	3Test(1.5 mL) 10Tests (5 mL)	Yellow Transparent Liquid	-5~-20℃, 1 year
Special Separation Solution A For Mouse Lliver Sinusoidal Endothelial Cells	3Tests (20 mL) 10Tests (75 mL)	Colorless Transparent Liquid	2-8℃, 1 year
Special Separation Solution B For Mouse Liver Sinusoidal Endothelial Cells	3Tests (20 mL) 10Tests (75 mL)	Colorless Transparent Liquid	2-8℃, 1 year
Special Separation Solution C For Mouse Liver Sinusoidal Endothelial Cells	3Tests (10 mL) 10Tests (30 mL)	Colorless Transparent Liquid	2-8℃, 1 year
Basic Culture Medium For Mouse Liver Sinusoidal Endothelial Cells	3Tests (100 mL) 10Tests (300 mL)	Red Transparent Liquid	2-8℃, 1 year

Continued

Name	Size	Appearance	Storage and Expiration Date
Supplements For Mouse Liver Sinusoidal Endothelial Cells	3Tests (10 mL) 10Tests (30 mL)	Yellow Transparent Liquid	-5~-20℃, 1 year
70 μm Cell Filter	3Tests (3 pcs) 10Tests (10 pcs)	Orange	Room temperature, 3 years
100 μm Cell Filter	3Tests (3 pcs) 10Tests (10 pcs)	Green	Room temperature, 3 years

Note:All components should be stored according to the temperature indicated on the labels of the reagent tubes. Specific Digestive Enzyme For Mouse liver Sinusoidal Endothelial Cells can be preserved at 4℃ for 30 days after thawing. For long-term storage, aliquot them into single-use portions and freeze at -20℃. Thaw again 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 mice to familiarize operators with procedural workflows and improve tissue dissociation efficiency.
2. During the entire tissue dissociation process, place the small dish containing the tissue on an ice tray/ice box (2-8℃) to maintain hypothermic conditions. Critical precautions: Monitor temperature rigorously to prevent ice crystal formation in tissues/liquids.
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 or contamination.
4. All special separation solution in this kit may exhibit crystal precipitation at the bottle mouth and mild turbidity during storage. These phenomena are normal and do not affect the performance or usability of the reagents.

Operational Procedures

1. Pre-experiment Preparation

(1) Self-supplied Reagents and Consumables: Eppendorf (EP) tube racks; one ice tray/ice plate; Phosphate-Buffered Saline (PBS); surgical instruments (At least 3 pairs of ophthalmic scissors; 2 pair of straight forceps; 1 pairs of curved forceps); culture dishes; T25 culture flask; dissection board (foam board substitute): assorted 2 mL/15 mL/50 mL centrifuge tubes. For scale-up culture, the user is required to provide complete culture medium and trypsin solution

(2) Reagent Thawing and Rewarming:

a. **Specific Digestive Enzyme For Mouse liver Sinusoidal Endothelial Cells**,**Supplements For Mouse Liver Sinusoidal Endothelial Cells** : Thaw at 4℃ and equilibrate to room temperature.

b. **Specialized Washing Solution For Mouse Liver Sinusoidal Endothelial Cells**, **Diluent of Digestive Enzyme For Mouse Liver Sinusoidal Endothelial Cells**, **Special Separation Solution A For Mouse Liver Sinusoidal Endothelial Cells, Special Separation Solution B For Mouse Liver Sinusoidal Endothelial Cells**, **Special Separation Solution C For Mouse Liver Sinusoidal Endothelial Cells**, **Basic Culture Medium For Mouse Liver Sinusoidal Endothelial Cells**: Equilibrate to room temperature.

c. Preparation of **Complete Culture Medium For Mouse Liver Sinusoidal Endothelial Cells**: Add 5 mL of **Supplements For Mouse Liver Sinusoidal Endothelial Cells** into 50 mL of **Basic Culture Medium For Mouse Liver Sinusoidal Endothelial Cells**, mix thoroughly.

d. Preparation of **Digestive Working Solution For Mouse Liver Sinusoidal Endothelial Cells**: Add 0.5 mL **Specific Digestive Enzyme For Mouse Liver Sinusoidal Endothelial Cells** into 4.5 mL **Diluent of Digestive Enzyme For Mouse Liver Sinusoidal Endothelial Cells**, mix thoroughly.

Note: Complete culture medium: 2-8℃, valid for 3 months. When preparing complete culture medium, it can be prepared according to the usage amount. Remaining supplements should be aliquoted proportionally and stored at -20℃ to avoid repeated freeze-thaw cycles.

2. Dissection Protocol

(1) Animal Sterilization and Euthanasia: 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) Preparation:

a. Preparation: Arrange sterilized scissors and forceps in pairs (ophthalmic scissors and straight/curved forceps) from left to right on two sterilized EP tube racks: Ophthalmic Scissors 1 and Straight Forceps 1; Ophthalmic Scissors 2 and Straight Forceps 2; Ophthalmic Scissors 3 and Curved Forceps 3.

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.

b. Mouse fixation: Secure the mouse in a prone position within the clean bench using needles for stabilization during tissue harvesting.

(3) Use Straight Forceps 1 to fix and clamp the skin of the abdomen and chest. Use ophthalmic scissors 1 to longitudinally cut the skin of the open chest along the midline of the abdomen. Use Straight Forceps 1 to clamp the skin along the opening. Use ophthalmic scissors 1 to horizontally cut the skin in the direction of the limbs. Use ophthalmic scissors 1 to cut and scrape off the adhesion between the skin and subcutaneous tissue. Be careful not to cut the peritoneum. Use Straight Forceps 1 to peel off the skin and pull it to both sides, exposing the superficial muscle layer of the abdominal wall.

(4) Fix and clamp the peritoneum with Straight Forceps 2, and cut open the peritoneum with Ophthalmic Scissors 2 to fully expose the liver tissue. Fix the liver tissue with Straight Forceps 2, cut off the connective tissue and blood vessels around the liver with Ophthalmic Scissors 2, remove the intact liver tissue, and place the tissue in a glass culture dish (place the entire culture dish on an ice tray/ice box to maintain a low temperature environment). Add an appropriate amount of **Specialized Washing Solution For Mouse Liver Sinusoidal Endothelial Cells** to the culture dish in advance and immerse the tissue.

3. Tissue Processing and Digestion

(1) Tissue Processing

a. Using Curved Forceps 3, clamp the junction of the liver lobes and gently shake the liver tissue. At this point, blood stains flow out. discard the liquid in the dish and add new **Specialized Washing Solution For Mouse Liver Sinusoidal Endothelial Cells** until the tissue is completely immersed. Rinse the tissue 2-3 times and use Ophthalmic Scissors 2 and Curved Forceps 3 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 and 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 3). add **Specialized Washing Solution For Mouse Liver Sinusoidal Endothelial Cells** from the upper side wall of the dish until it just immersed the tissue at the bottom of the dish (tilt the dish about 45° throughout the entire process).

b. 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 Mouse Liver Sinusoidal Endothelial 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.

(2) Tissue Digestion

a. Add 5 mL of **Digestive Working Solution For Mouse Liver Sinusoidal Endothelial Cells** to the 50mL centrifuge tube tissue sediment, blow and mix well, wrap the centrifuge tube mouth with a sealing film, and place the tube horizontally at 4℃ for 16 hours of digestion (digestion time should not exceed 16 hours, as excessive digestion will have a significant impact on cell activity).

b. After digestion, remove the centrifuge tube and transfer it to a 37℃ water bath shaker at 150 rpm for 15 minutes of digestion..

4. Cell Isolation

(1) 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 washing solution.

(2) Carefully blow the tissue suspension in the previous 50 mL centrifuge tube with a 5 mL pipette or a Pasteur pipette. After filtering through a **100 μm Cell Filter** and a **70 μm Cell Filte**r in turn, slowly add 3-5 mL of **Specialized Washing Solution For Mouse liver Sinusoidal Endothelial Cells** to the top of the filter with a clean pipette tip to collect the tissue digestion suspension on the filter. Collect the filtrate in a 50 mL centrifuge tube, centrifuge the cell filtrate at 400 g for 10 minutes, discard the supernatant, and retain the precipitate.

Note: Filtration standard - The suspension can easily pass through the filter without a large amount of colloidal substance. 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 filter and the centrifuge tube mouth. At this time, you can try tilting the cell filter slightly and placing it on a 50 mL centrifuge tube to improve this problem.

(3) Resuspend the cell pellet in a 50 mL centrifuge tube using 6 mL of **Special Separation Solution A For Mouse Liver Sinusoidal Endothelial Cells**. Centrifuge at 900 g for 15 minutes and discard the supernatant (which contains liver stellate cells, a small amount of sinusoidal endothelial cells, Kupffer cells, hepatocytes, etc.) retaining the precipitate.

(4) Resuspend the precipitate obtained from the 50 mL centrifuge tube in the previous step using 6 mL of **Special Separation Solution B For Mouse Liver Sinusoidal Endothelial Cells**. Take a new sterile 15 mL centrifuge tube and add 3 mL of **Special Separation Solution C For Mouse Liver Sinusoidal Endothelial Cells**. Use a 1 mL pipette to collect the cell suspension from the 15 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 Mouse Liver Sinusoidal Endothelial 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 draw 50mL of resuspended centrifuge tube 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 separation solutions. When adding the cell suspension to the specialized separation solution C for mouse sinusoidal endothelial cells, the action should be gentle and slow, ensuring that the suspension is above the Special Separation Solution C and avoiding mix the two separation solutions, which may affect the subsequent reduction of layered cell volume.

(5) 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 2-5 mm thick liver stellate cells,the second layer is approximately 6 mL separation solution B, the third one is approximately 2-3 mm thick liver sinusoidal endothelial cells and 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).

(6) 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 Mouse Liver Sinusoidal Endothelial Cells** to resuspend the cell suspension, centrifuge at 400 g for 5 min, discard the supernatant, and retain the precipitate.

5. Cell Culture and Subculture

(1) Cell seeding: Resuspend 5 mL of **Complete Culture Medium For Mouse Liver Sinusoidal Endothelial Cells** and seeding it into a new 6 cm culture dish A. Incubate in a 5% CO_2 incubator at 37℃ for 20 minutes, then transfer the supernatant A to a new culture dish B. Add 5 mL of fresh **Complete Culture Medium For Mouse Liver Sinusoidal Endothelial Cells** to the original culture dish A and continue culturing.

(2) After the supernatant A in culture dish B was incubated at 37℃ and 5% CO_2 for 15 minutes, the supernatant B in the dish was transferred to a new T25 cell culture flask. The original culture dish B was further cultured with 5 mL of fresh **Complete Culture Medium For Mouse Liver Sinusoidal Endothelial Cells**.

Note: This step involves differential separation of different cell attachment. In dish A, the remaining cells are mainly liver Kupffer cells with strong attachment (more dispersed, spindle shaped macrophage like), while in dish B, the remaining cells are mainly liver Kupffer cells and a small amount of sinusoidal endothelial cells. Observing these two dishes of cells will help to identify potential issues that may arise in the future. The T25 flask contains high-purity sinusoidal endothelial cells.

(3) Medium replacemen: After the supernatant B in culture dish B was incubated at 37℃ and 5% CO_2 for 24 hours, followed by subsequent replacements every 2-3 days.

(4) Cell passaging protocol:The mouse liver sinusoidal endothelial Cell can be passaged 1-2 times. After about 3 days of inoculation, when cells reach 80-90% confluency, show a spindle shaped endothelial like trend of aggregation, which can be passaged. 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 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℃ incubator for 1-3 minutes. After most of the cells round up and detach, add 5 mL of complete culture medium for mouse sinusoidal endothelial cells to terminate digestion. 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℃ in a humidified atmosphere with 5% CO_2 and saturated humidity.

Troubleshooting

Table.2.Common problems, causes, and solutions of liver sinusoidal endothelial separation in mice

Problem	Possible Cause	Solution
A large amount of tissue block are deposited at the bottom of the tube (procedure3-step(2).a)	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,The volume of digestive fluid is greater than or equal to three times that of the tissue block.
After rewarming digestion, the tissue cannot be completely blown away, and there are still block like tissues （procedure3-step(2)）	Inadequate digestion (improper storage conditions for digestive enzymes, enzyme inactivation; inadequate tissue digestion)	Reopen a bottle of digestive enzyme and store it according to the requirements; Grinding tissue can be considered without influence cells activ
No obvious 5 layers were observed after centrifugation of the separation system（procedure4-step(5)）	During the layering operation, two liquids merge and a small amount of layering occurs.	Strictly follow the centrifugal acceleration and deceleration requirements in step (5) of process 4 to set the centrifugal speed
No white membrane layer was observed in the target layer after centrifugation of the separation system（procedure4-step(5)）	Overdigestion leads to cell death	Control the digestion time at 4 ℃ to not exceed 16 hours
Low cell quantity	The volum of tissue obtain is few，Inadequate differential attachment time	Strictly follow the instructions for Mouse age and number (obtaining too little liver tissue may increase the number of extracted Mouses); 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	There are kupffer cells mixed in the cell fluid..	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).

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Anatomy Images for Reference

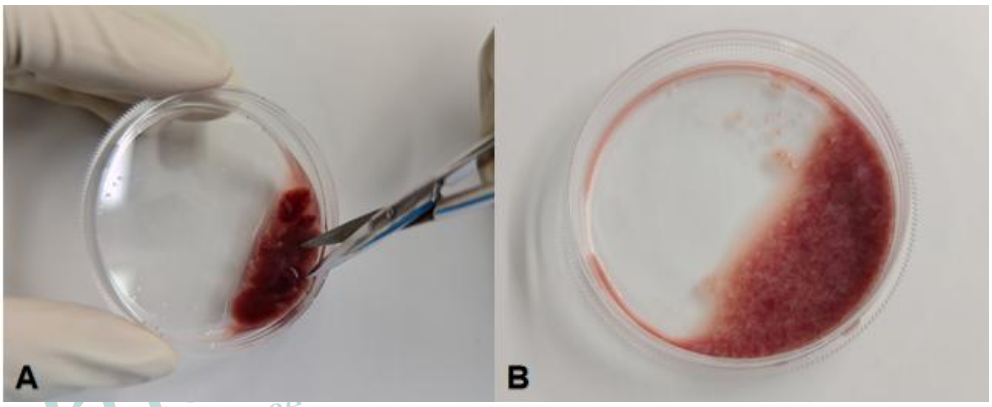


Figure 1. Treatment of liver tissue in vitro
A: Demonstration of liver tissue cutting operation; B: liver after cutting into pieces

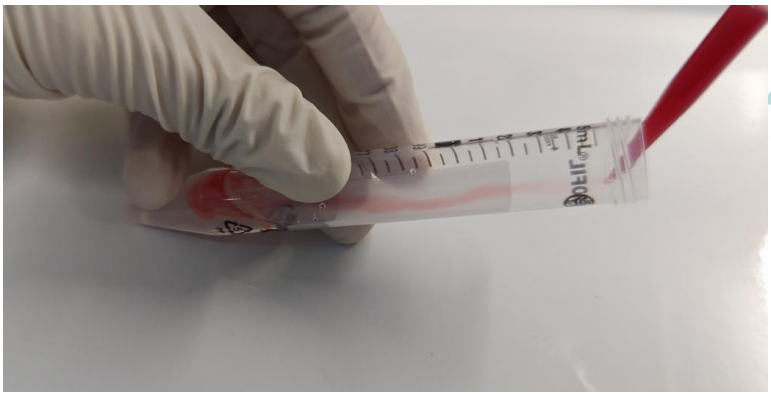


Figure 2. Demonstration of Layered Operation of Cell Suspension and Separation solution

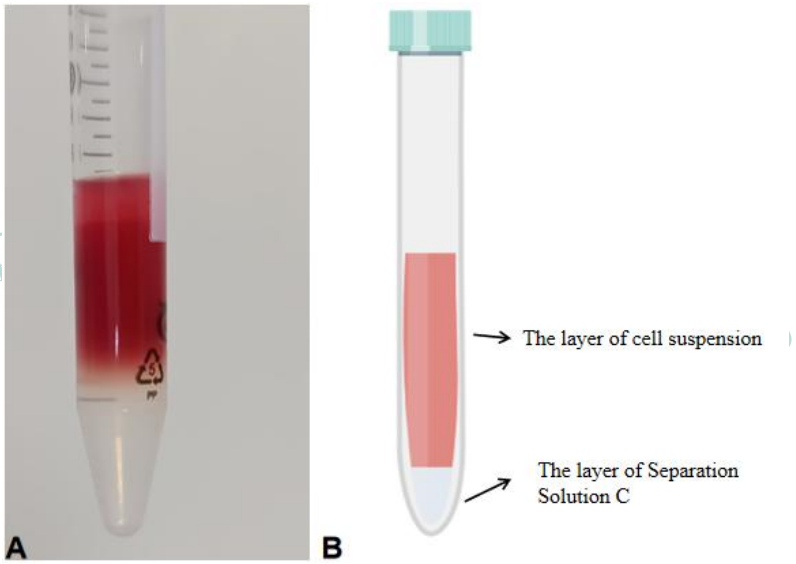


Figure 3. Reference diagram for liquid level stratification
A: Real photos of cell suspension and separation solution C after stratification;
B: Schematic diagram of cell suspension and separation solution C after stratification

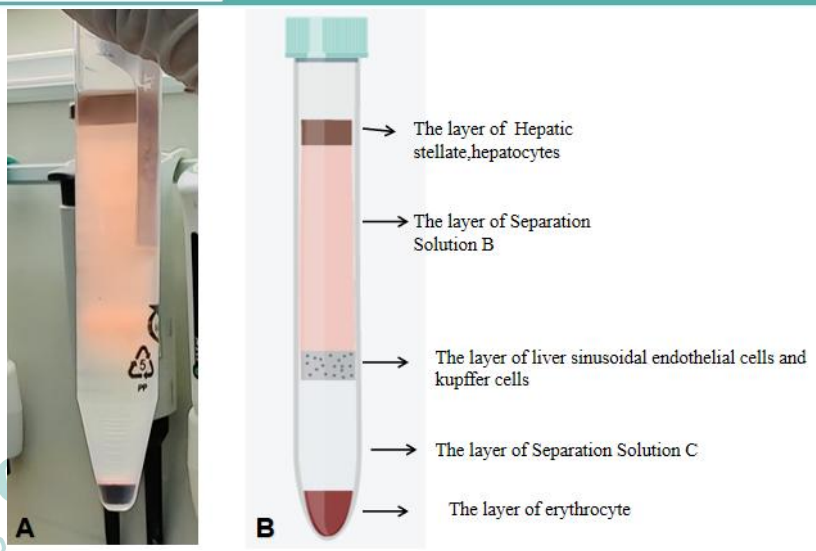


Figure 4. Reference image of cell suspension after centrifugation with separation solution
A: Cell suspension was centrifuged with separation solution and layered in real photos;
B: Schematic diagram of layering of cell suspension after centrifugation with separation solution
(For reference only, the thickness of the cell layer and the color depth of the separation solution B layer may vary depending on experimental conditions)

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