Rat Pulmonary Aorta Smooth Muscle Cells Isolation and Culture Kit

Cat. No.: P-CA-609

Size: 3Tests/10Tests

Background

The Rat Pulmonary Aorta Smooth Muscle Cells Isolation and Culture Kit is specifically developed for the extraction of primary rat pulmonary aorta smooth muscle Cells. Verified through standardized procedures, each 1 Test of 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 subcultured at a 1:2 ratio, the cells can undergo 5 passages,with the best cell state within the first 3 passages.Through immunofluorescence analysis, the cell purity (α-SMA-positive rate) has been confirmed to exceed 90%.

Scope of Application

This product is suitable for extracting smooth muscle cells from the pulmonary artery of rats from various strains (e.g., Wistar, SD), ranging in age from 20 to 30 days. Following tissue isolation, enzymatic digestion, and 72-hour plating purification, a yield exceeding 1×10^6 cells can be obtained.

Note: The intact pulmonary artery tissue extracted from 8 rats (with tissue yield of each hind limb shown in Figure 11a) typically yield enough cells for one T-25 flask. The specific number of rats required may vary depending on the completeness and quantity of the pulmonary artery obtained during this procedure. If the amount of tissue obtained is insufficient, additional experimental rats may be needed to prevent cell quantity deficiency.

Kit Components

Name	Size	Appearance	Storage and Expiration Date
Specialized Washing Solution For Rat Pulmonary Aorta Smooth Muscle Cells	3Tests (250 mL) 10Test (500 mL×2)	Pale Yellow Transparent Liquid	2-8℃,1 year
Specialized Digestive Solution For Rat Pulmonary Aorta Smooth Muscle Cells	3Tests (15 mL) 10Tests (50 mL)	Yellow Transparent Liquid	-5~-20℃,1 year
Basic Culture Medium For Rat Pulmonary Aorta Smooth Muscle Cells	3Tests (50 mL) 10Tests (100 mL)	Red Transparent Liquid	2-8℃, 1 year
Supplement For Rat Pulmonary Aorta Smooth Muscle Cells	3Tests (5 mL) 10Tests (10 mL)	Yellow Transparent Liquid	-5~-20℃,1 year

Note:All components should be stored according to the temperature indicated on the labels of the reagent tubes. The reagents stored at -5~-20℃ (such as Specialized Digestive Solution and supplement for Rat Pulmonary Aorta Smooth Muscle 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.

Note

1. Prior to formal experiments, it is recommended to conduct anatomical simulation training using 1-2 normal rats to familiarize yourself with operational procedures and improve tissue isolation efficiency.It is difficult to obtain materials from the pulmonary artery, so it is necessary to practice to find the position of the pulmonary artery before starting the formal experiment.
2. Reagent preparation or aliquoting must strictly adhere to aseptic techniques. After dispensing, seal the containers immediately with a sealing film, use them promptly to avoid repeated freeze-thaw cycles or contamination.

Operational Procedures

1. Pre-experiment Preparations

1) Self-supplied Reagents and Consumables: two Eppendorf (EP) tube racks; ;Phosphate-Buffered Saline (PBS); surgical instruments (At least 3 pairs of ophthalmic scissors; 1 pair of straight forceps; 2 pairs of curved forceps; 1 pair of micro straight forceps; 1 pair of micro curved forceps; 1 pair of micro scissors); 6 cm/10 cm culture dishes; T25 culture flasks; 6-well plate;dissection board (foam board can substitute): and multiple 2 mL/15 mL/50 mL centrifuge tubes.

2) Reagent Thawing and Rewarming:
   ① Specialized Digestive Solution For Rat Pulmonary Aorta Smooth Muscle Cells&Supplement For Rat Pulmonary Aorta Smooth Muscle Cells: Thaw at 4℃ and equilibrate to room temperature.
   ② Specialized Washing Solution For Rat Pulmonary Aorta Smooth Muscle Cells&Basic Culture Medium For Rat Pulmonary Aorta Smooth Muscle Cells: Equilibrate to room temperature.

3) Preparation of Complete Culture Medium: Add 5 mL of Supplement For Rat Pulmonary Aorta Smooth Muscle Cells into 50 mL of Basic Culture Medium For Rat Pulmonary Aorta Smooth Muscle Cells, mix thoroughly.

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

2. Dissection Procedures

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

2) Dissection and Tissue Harvesting Steps:
   ① Preparation: Arrange sterilized scissors and forceps in pairs in order of use from left to right on two sterilized EP tube racks (Ophthalmic Scissors 1 and Straight Forceps 1, Ophthalmic Scissors 2 and Curved 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.

   ② Rat Fixation: Secure the rat in a supine position within the clean bench using needles, preparing for tissue harvesting.

3）Tissue Harvesting Procedure:
   ① Using Straight Forceps 1 to grasp the upper abdominal skin, cut the skin from the upper abdomen to the neck, and cut the skin on both sides upward to the neck using Ophthalmic Scissors 1.Until the sternum is fully exposed.

   Note: Cut the skin to expose the entire chest, taking care to tear the hair away from the anatomical area to prevent contamination.

   ② Hold the right inferior rib arch of the rat with Curved Forceps 2, and use Ophthalmic Scissors 2 to cut upward from the rib to the clavicle. Cut horizontally through the diaphragm, and cut along the left inferior rib to the left shoulder clavicle area. Cut the sternal handle, flip it upward, and fully open the thoracic cavity to expose the heart and lung tissues.

   Note: Do not insert the scissors too deep into the thoracic cavity. Cut lightly upward and forward, and do not Cutting through lung tissue can easily cause contamination.

   ③ Turn the dissection board and head the rat towards yourself. The left hand uses the Curved Forceps 3 to pull the thymus away to avoid obstructing the line of sight.Then use the Curved Forceps 3 to hold the atrium and gently lift it up, find the pulmonary artery originating from the right ventricle and the aortic arch.Use the Ophthalmic Scissors 3 slightly to the left, cut down from the top of the blood vessel to avoid cutting into the esophagus, and cut off a mass of tissue containing the pulmonary artery (Including the

heart, aortic arch, and a small portion of lung tissue connected to the pulmonary artery). Put it into a culture dish containing 10mL Specialized Washing Solution For Rat Pulmonary Aorta Smooth Muscle Cells (Figure 1).

Note: Rat Pulmonary Aorta sampling is difficult.. It is recommended to first understand the growth position and course of the pulmonary trunk in the body, find the correct location before cutting, to avoid taking the wrong tissue.

3. Tissue Processing and Digestion

1) Tissue Processing

① Put Micro Straight Forceps, Micro Curved Forceps and Micro Scissors on the EP tube rack within the clean bench, ensuring the distal third of each tool suspended.

② Tissue purification with the new micro forceps set. The right hand used curved micro forceps to wash the tissue back and forth in the Specialized Washing Solution For Rat Pulmonary Aorta Smooth Muscle Cells to remove blood stains. Put it into a culture dish containing 10mL Specialized Washing Solution For Rat Pulmonary Aorta Smooth Muscle Cells.

③ Use the Micro Straight Forceps in the left hand and Micro Scissors in the right hand to cut off the bottom half of the heart (Figure 2).Use the right hand to squeeze and remove the blood clot from the blood vessel with the Micro Curved Forceps, and place the cleaned tissue into another new petri dish containing 10 mL of Specialized Washing Solution for Rat Pulmonary Artery Smooth Muscle Cells for further use (Figure 3).

Note：The excised vascular tissue has a lot of blood stains, which can be washed twice to remove most of the blood stains, so as to avoid the cleaning solution becoming too turbid during subsequent separation of the target blood vessels, affecting the field of view.

④ Prepare two petri dishes each containing 10 mL of specialized washing solution for rat pulmonary artery smooth muscle cells.Using the left hand to hold Micro Straight Forceps and the right hand to hold the Micro Curved Forceps, pick up a tissue and clean it within one of the petri dishes.First locate the aortic arch and pulmonary artery, then gently remove the pale yellow fat between the two vessels.(Figure 4)

⑤ Along the direction of blood vessel growth, locate the pulmonary trunk branch and clamp it with Micro Straight Forceps (Figure 5). Gently detach the branch from surrounding tissues using the Micro Curved Forceps (Figure 6).Then, using the Micro Scissors, the aortic arch and pulmonary artery tissues were separated from the heart at the root of the blood vessels (Figure 7), and the yellowish-pink adipose tissue on the blood vessels was removed (Figure 8).

⑥ Use the Micro Scissors to cut open the aortic arch and pulmonary artery (Figure 9), remove excess adipose tissue, and obtain pure pulmonary artery tissue (Figure 10).

⑦ Place the cleaned tissue into another new petri dish containing 10 mL of specialized washing solution for rat pulmonary artery smooth muscle cells. Cut the pulmonary artery tissue longitudinally (Figure 11).The left hand uses the Micro Straight Forceps to fix the cleaned tissue.The inner membrane was facing up, and the back of the Micro Curved Forceps hook was used to gently scrape the inner membrane surface dozens of times with the right hand to remove the endothelial cells. then carefully remove the obvious outer membrane layer.

Note：The force of the whole process should be gentle to avoid excessive pulling on blood vessels and affecting cell activity. The inner membrane layer of blood vessels is invisible to the naked eye, and the purpose of repeated scraping of the inner membrane surface is to destroy endothelial cells.

2) Tissue Digestion

① Place the purified pulmonary artery smooth muscle tissue into a 6 cm culture dish containing 5mL digestive fluid for rat pulmonary artery smooth muscle cells. Use the Micro Straight Forceps in the left hand to clamp the tissue, and use the Micro Scissors in the right hand to cut the tissue into small pieces of

about 5mm^2 (Figure 12),The petri dish was placed in the 37℃ incubator and digested overnight for 16-18 hours.

② After digestion, remove the petri dish from the incubator and use a 5 mL pipette or a Pasteur pipette to Pipette the suspension approximately 30 times to disperse the large tissue visible to the naked eye. After mixing thoroughly, add 5 mL Specialized Washing Solution for Rat Pulmonary Aorta Smooth Muscle Cells to the petri dish.

Note: The digestion time is determined according to the actual digestion effect. It can be observed under the microscope. There will be obvious circular cell arrangement on the freshly digested tissue block, and some cells will be free in the digestion solution. A small number of cell clusters and fragments will still be present after mixing with blowing, which is a normal phenomenon.

3) Cell Isolation

① Transfer the cell suspension to a 15 mL centrifuge tube and centrifuge at 1200 rpm for 5 min. Discard the supernatant and retain the cell pellet.

4. Cell Culture and Subculture

1) Cell Seeding: Take out the T25 cell culture flask, and resuspend the cell pellet in the centrifuge tube with 5 mL of 5 mL Complete Culture Medium of Pulmonary Aorta Smooth Muscle Cells , then inoculat into the T25 cell culture flask. The cells were cultured in a incubator at 37℃, 5% CO_2.(Approximately 1×10^6 cells can be obtained)

2) Medium replacement: Perform the first medium replacement at 48 h, followed by subsequent replacements every 2-3 days. Cells typically reach 80-90% confluency within 2-3 days post-seeding.

3) Cell Subculture: When the cell confluence reaches 80-90%, it is ready for passaging. First, aspirate and discard the medium from the T25 cell culture flask and wash the cells once with 2-3 mL PBS. Then, add 1mL of 0.25% trypsin digestive solution to the T25 flask, gently rotate the flask until the digestive solution covers the entire bottom, then aspirate and disgard the excess trypsin solution, incubate at 37℃ for 1-3 min. Next, observe under an inverted microscope until the cells retract and become rounded, then add 5mL of Complete Culture Medium for Rat Saphenous Vein Smooth Muscle Cells to terminate the digestion. Resuspend and disperse the cells by gently pipetting with a 5 mL pipette or Bacto pipette. Inoculate the cells into new culture vessels according to the split ratio or experimental requirements. Incubate them statically in a cell culture incubator at 37℃, 5% CO_2, and saturated humidity

Troubleshooting

Problem	Possible Cause	Solution
Low yield/low viability	Insufficient dissociation	Check the storage conditions of the digestion solution to ensure it has not been stored at 4℃ for more than 30 days
		Ensure the tissue quantity matches the kit requirements
		Make sure the smooth muscle tissue is not cut too large
		Ensure that the tissue is gently and adequately pipette dup and down.
	Over-digestion	Strictly control the size of the tissue block to avoid cutting too small
Slow cell growth	Improper preparation of culture medium	Prepare complete culture medium with accurate ratios and avoid repeated freeze-thaw cycles
		Use complete culture medium within its validity period and avoid preparation older than three months
	Over-aged rats	Use rats aged 20-30 days postnatally to prevent slower cell proliferation and reduced passage numbers associated with cells extracted from older rats.

	Improper subculturing ratio	When passaging at 1:2 ratio, calculate based on vessel surface area to maintain proper cell seeding density
	Over-passaged	Limit cell passage to 3-5 times to prevent a slowdown in proliferation.
	shortage of tissue sampling amount	If the tissue amount of rat pulmonary aorta is small, the rat amount can be increased appropriately
Low cell purity	The outer membrane layer of the tissue was not completely removed	Make sure the blood vessels are cleaned
	The endothelial cells were not completely destroyed	Make sure all areas of the inner lining are scraped back and forth dozens of times

Anatomy Images for Reference

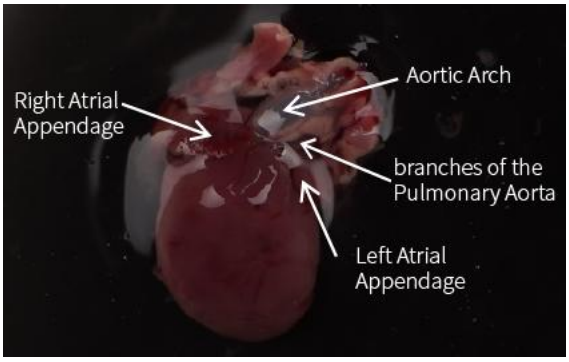

Figure1.Cut off a mass of tissue containing the pulmonary artery

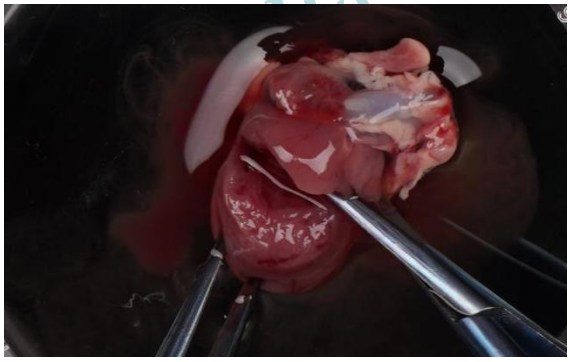

Figure 2. Cut off the bottom half of the heart

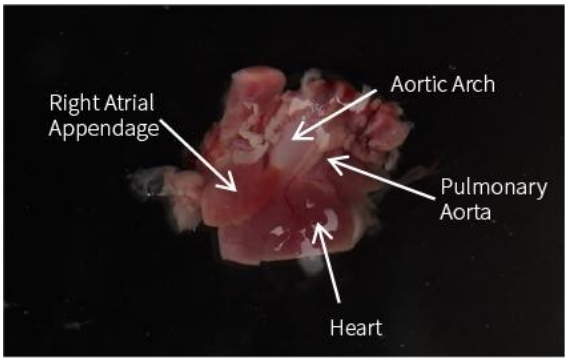

Figure 3. The blood clot-cleared tissue mass

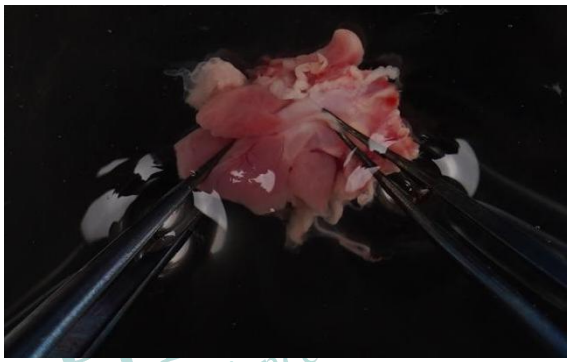

Figure 4a. Remove the fat between the aortic arch and the pulmonary artery

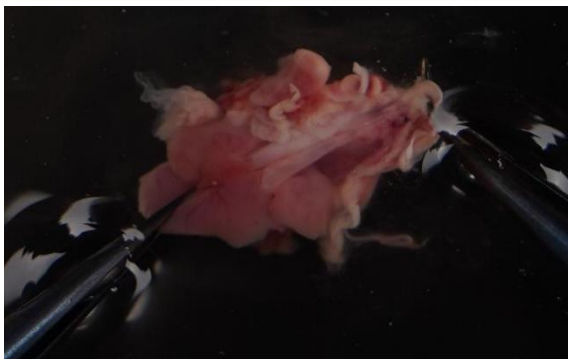

Figure 4b Remove the fat between the aortic arch and the pulmonary artery

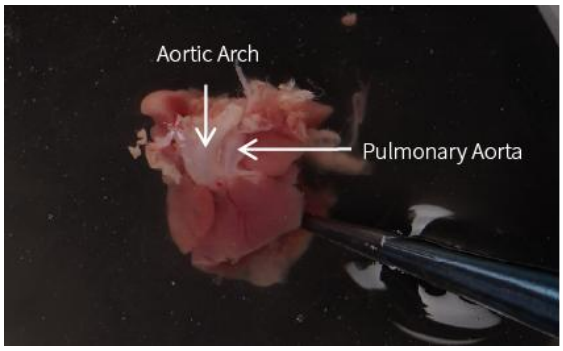

Figure 5 Find the pulmonary artery and clamp it with a straight micro forceps

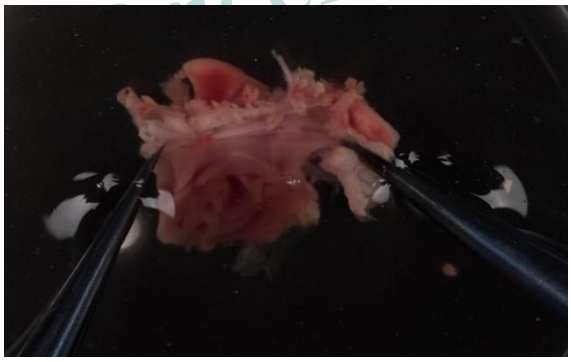

Figure 6a Gently detach the branch from surrounding tissues using a micro curved forceps

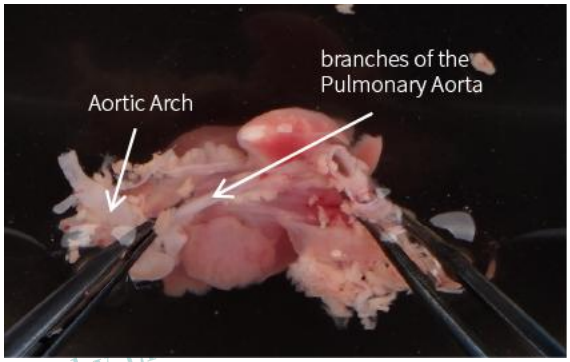

Figure 6b Gently detach the branch from surrounding tissues using a micro curved forceps

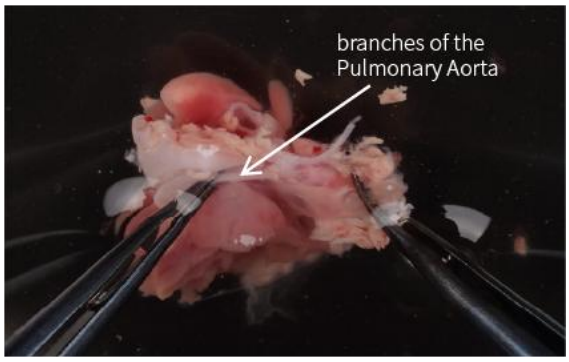

Figure 6c Gently detach the branch from surrounding tissues using a micro curved forceps

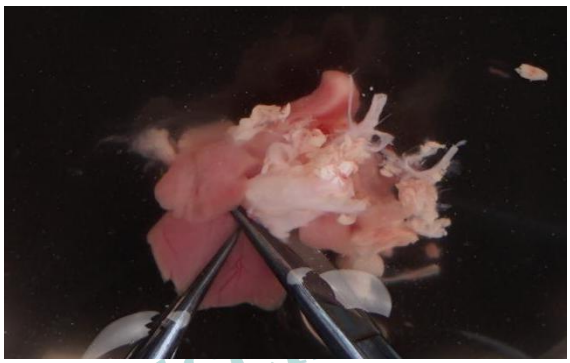

Figure 7a Cut along the location where the blood vessels are connected to the heart.

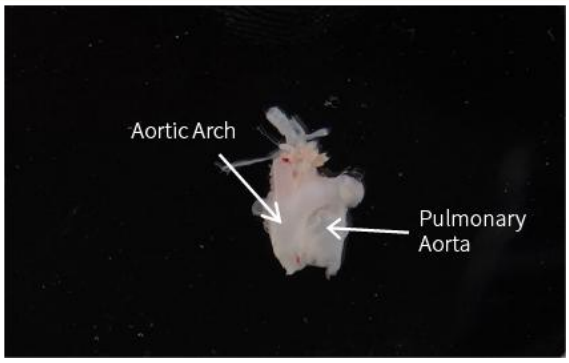

Figure 7a aortic arch and pulmonary artery tissue

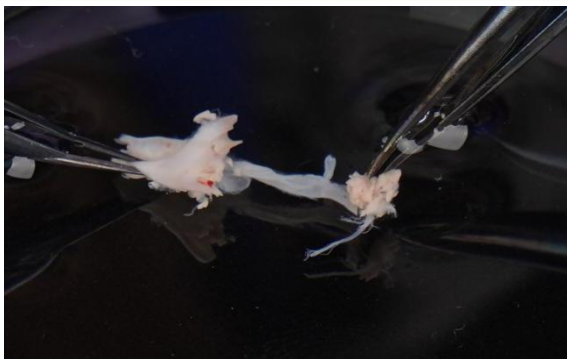

Figure 8 Remove the yellowish pink fat from the blood vessels

Figure9 Cut open two blood vessels

Figure10 Remove excess fat and outer membrane tissue

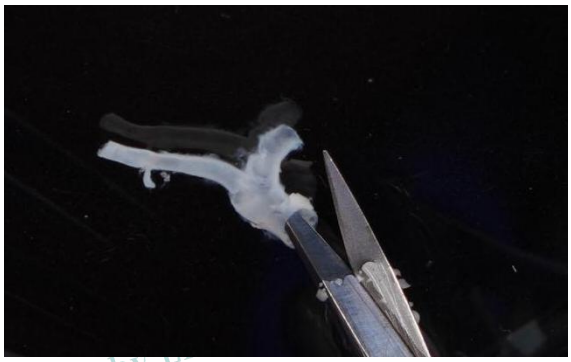

Figure11a Cut the pulmonary artery tissue longitudinally

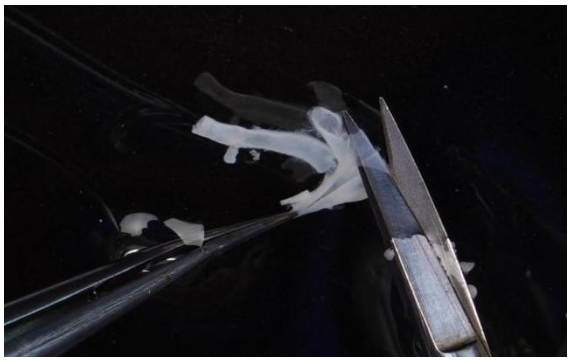

Figure11b Cut the pulmonary artery tissue longitudinally

Figure11c Cut the pulmonary artery tissue longitudinally

Figure11d Cut the pulmonary artery tissue longitudinally

Figure12a Cut the pulmonary artery tissue into pieces

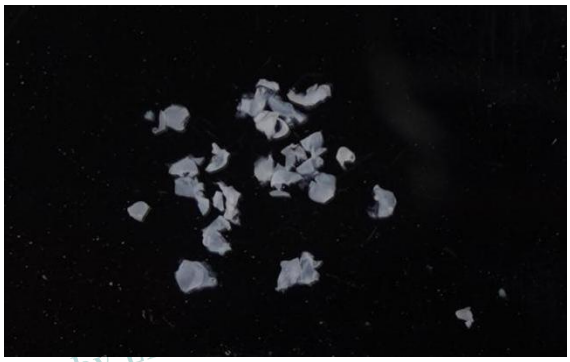

Figure12b Cut the pulmonary artery tissue into pieces