High Activity Tumor Tissue Enzymatic Digestion Kit (Mouse) Instructions

Product Information

Product Name	Model	Specification
High Activity Tumor Tissue Enzymatic Digestion Kit (Mouse)	DHTE-5001	50 T

Product Description

High Activity Tumor Tissue Enzymatic Digestion Kit (Mouse) (the "Kit") can prepare tumor tissue of mouse into single cell suspension gently, quickly and efficiently. This optimized protocol can help obtain as many single cell samples with high cell viability as possible, while maintaining the important surface epitopes of cells. The single cell suspension can be applied in downstream experiments such as cell sorting, primary cell culture of tumor cell or tumor infiltrating lymphocytes (TIL), and single cell sequencing.

Main principle: Mechanical dissociation is in combination with enzymatic digestion of the extracellular matrix (while maintaining the intactness of tissue structure) to prepare tumor tissue into single cell suspension. RWD Single Cell Suspension Dissociator is chiefly used for mechanical dissociation, while the Kit mainly digests the tissue through enzymatic digestion. After dissociation, the cell suspension is filtered through the cell strainer to remove tissue residues to obtain single cell suspension.

Components

Product Name	Components	Quantity	Storage Condition
High Activity Tumor Tissue Enzymatic Digestion Kit (Mouse)	Enzyme A Reagent (powder)	1 vial	2℃ ~ 8℃
	Enzyme B Reagent (powder)	1 vial	2℃ ~ 8℃
	Enzyme C Reagent (powder)	1 vial	-25℃ ~ -15℃
	Buffer B (solution)	1 vial	2℃ ~ 8℃
	Buffer C (solution)	1 vial	2℃ ~ 8℃

Test Capacity

Recommendation for single process of tissue:

Tissue Type	Capacity	Initial Sample Dosage
Mouse Tumor Tissue	50 T	10 ~ 1000 mg to be processed per time

Storage & Transportation

- ✧ Transported at 2℃ ~ 8℃.
- ✧ The Kit is separated into two packages due to different storage temperature, please store them separately according to the attached temperature label.
- ✧ It is recommended that all the enzyme reagents should be dissolved separately, mixed evenly and stored in small packages. Avoid repeated freezing, thawing and shaking.
- ✧ The Kit is valid for 12 months from the date of manufacture.

Reagent & Instrument

Reagent	RPMI 1640 or DMEM Medium	Red Blood Cell Lysis Buffer (optional)	
Consumable	Tissue Processing Tube (RWD)	Heater (RWD # HJ-400)	100 μm Cell Strainer
	0.22 μm Syringe Filter (optional)		
Instrument	Single Cell Suspension Dissociator (RWD)	Constant Temperature Oscillator	

Operation

Preparation

(1) Preparation of enzyme A solution: Dissolve the powder of the enzyme A reagent with 2.75 mL RPMI 1640 or DMEM medium, subpakcage the solution and store at -25℃ ~ -15℃. The enzyme solution can be stored stably for 6 months at -25℃ ~ -15℃ and it should avoid repeated freezing and thawing.

(2) Preparation of enzyme B solution: Dissolve the powder of the enzyme B reagent with 6.875 mL buffer B, subpakcage the solution and store at -25℃ ~ -15℃. The enzyme solution can be stored stably for 6 months at -25℃ ~ -15℃ and it should avoid repeated freezing and thawing.

(3) Preparation of enzyme C solution: Centrifugate the enzyme C powder to the tube bottom by instantaneous centrifugation in a palm microcentrifuge. Dissolve the powder of the enzyme C reagent with 1.375 mL buffer C, subpakcage the solution and store at -25℃ ~ -15℃. The enzyme solution can be stored stably for 6 months at -25℃ ~ -15℃ and it should avoid repeated freezing and thawing.

(4) Preparation of enzyme mixture:

Prepare the enzyme mixture 1 according to the table below, and the enzyme mixture should be freshly prepared just before use. The enzyme mixture 1 can be used for processing 10 ~ 1000 mg mouse tumor tissue. When processing tumor tissue greater than 1000 mg, the volume of enzyme mixture should be increased in proportion. A tissue processing tube can be used to process up to 1000 mg tumor tissue that the amount of tissue processing tube should be increased if more tissue is to be processed. If subsequent cell culture is required, the enzyme mixture 1 needs to be sterile-filtered by the 0.22 μm syringe filter and the volume of the filtered mixture should be 2.5 mL in total.

Enzyme Mixture 1			
RPMI 1640 or DMEM Medium 2300 μL	Enzyme A 50 μL	Enzyme B 125 μL	Enzyme C 25 μL

⚠ Note: If tumor infiltrating lymphocytes (TIL) are to be analyzed, the volume of enzyme B should be adjusted. Prepare the enzyme mixture 2 according to the table below, and the enzyme mixture should be freshly prepared just before use. This formula can better protect the surface epitopes of cells, but will slightly decrease the yield of cells.

Enzyme Mixture 2			
RPMI 1640 or DMEM Medium 2400 μL	Enzyme A 50 μL	Enzyme B 25 μL	Enzyme C 25 μL

Mechanized Protocol

(1) After obtaining tumor tissue of mouse, cut the tissue into pieces of 2 ~ 4 mm, put them in the petri dish containing RPMI 1640 or DMEM medium for temporary storage, and weigh target weight of the tissue pieces by the electronic balance scale.

(2) Transfer the tissue pieces to the tissue processing tube containing enzyme mixture (mixture 1 or 2).

(3) Tighten the tissue processing tube, invert it and mount it in the bushing of the single cell suspension dissociator with the heater.

⚠ Note: Make sure the sample is in the area where the rotor/stator is located.

(4) For soft tumor tissue, run the program **M_Tumor_Heater_1**; for hard tumor tissue, run the program **M_Tumor_Heater_2**.

(Optional) For hard tissue, there may still remain some large tissue pieces in the tissue processing tube after the program **M_Tumor_Heater_2** is finished. It is suggested to blow the tissue pieces 10 ~ 15 times by the 1 mL pipette, which the tip is cut off 0.5 cm, to help release more single cells.

(5) After the program is finished, remove the tissue processing tube from the single cell suspension dissociator. Wet the 100 μm cell strainer with 1 mL RPMI 1640 or DMEM medium, filter the cell suspension through the wetted cell strainer, and collect the cell suspension to the 50 mL centrifuge tube.

(6) Rinse the tissue processing tube with 10 mL RPMI 1640 or DMEM medium, filter the suspension through the 100 μm cell strainer and collect it to the 50 mL centrifuge tube mentioned in step (5).

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(7) Centrifugate the cell suspension at 500×g for 5 min and discard the supernatant completely.

(Optional) Removal of red blood cells

If it is necessary to remove the red blood cells, resuspend the cells collected in step (7) with 1 ~ 2 mL red blood cell lysis buffer. Then, incubate the suspension on ice for 2 ~ 3 min, followed by resuspension with 10 mL RPMI 1640 or DMEM medium. Centrifugate the cell suspension at 500×g for 5 min and discard the supernatant completely.

(8) Resuspend the cell suspension with RPMI 1640 or DMEM medium or other buffer to required volume for subsequent experiment.

Manual Protocol

(1) After obtaining mouse tumor tissue, cut the tissue into pieces of 2 ~ 4 mm, put them in the petri dish containing RPMI 1640 or DMEM medium for temporary storage and weigh target weight of the tissue pieces by the electronic balance scale.

(2) Transfer the tissue pieces (both soft and hard tissues) to the tissue processing tube or centrifuge tube containing enzyme mixture (mixture 1 or 2) in step (4) of ***"Preparation"***.

⚠ Note: In manual protocol, tissue processing tube can be replaced by 50 mL centrifuge tube.

(3) Place the tissue processing tube or 50 mL centrifuge tube in the 37°C constant temperature oscillator, incubate the cell suspension at 150 rpm for 15 min and blow the tissue pieces 20 times by the 1 mL pipette, which the tip is cut off 0.5 cm. Then, repeat the incubation and blowing again.

(4) Place the tissue processing tube or 50 mL centrifuge tube again in the 37°C constant temperature oscillator, incubate the cell suspension at 150 rpm for 10 min and blow the tissue pieces 10 times by the 1 mL pipette.

(5) Follow the steps in ***"Mechanized Protocol"*** from step (5) to (8).

⚠ Note: Compard with mechanized protocol, the manual protocol has a certain fluctuation of cell number and incomplete digestion. It is suggested to adjust the time of digestion and blowing according to actual condition.

Precautions

(1) The Kit is valid for 12 months and RWD shall not guarantee the validity of expired products.

(2) For any downstream cell culture to be performed subsequent to tissue dissociation, it is necessary to ensure that all steps are performed under sterile conditions.

(3) Each 0.01 ~ 1.0 g tumor tissue processed requires approximately 2.5 mL enzyme mixture for enzymatic digestion.

(4) The Kit has passed the transportation test, so the performance of the Kit is not affected though the ice pack equipped with the Kit has melted upon receipt.

* Note: The tissue processing tubes of RWD are not available in the USA.

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