High Activity Whole Skin Enzymatic Digestion Kit (Mouse) Instructions

Product Information

Product Name	Model	Specification
High Activity Whole Skin Enzymatic Digestion Kit (Mouse)	DHWSE-2509	25 T

Product Description

High Activity Whole Skin Enzymatic Digestion Kit (Mouse) (the "Kit") can prepare back skin or ear skin tissue of mouse aged 6 ~ 10 weeks 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 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 skin 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 Whole Skin 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℃
	Buffer D (solution)	1 vial	2℃ ~ 8℃
	High Efficiency Debris Removal Reagent (solution)	1 vial	2℃ ~ 8℃

Test Capacity

Recommendation for single process of tissue:

Tissue Type	Capacity	Initial Sample Dosage
Mouse Back Skin or Ear Skin Tissue	25 T	20 ~ 500 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	DMEM Medium or RPMI 1640	PBS	HBSS Buffer (with Ca^{2+} and Mg^{2+})
Consumable	Tissue Processing Tube (RWD)	Heater (RWD: # HJ-400)	40 μm Cell Strainer
	0.22 μm Syringe Filter (optional)		
Instrument	Single Cell Suspension Dissociator (RWD)	High-Speed Benchtop Refrigerated Centrifuge (RWD: # M1416R)	Constant Temperature Oscillator

Operation

Preparation

(1) Preparation of enzyme A solution: Dissolve the powder of the enzyme A reagent with 2.7 mL HBSS buffer (with Ca^{2+} and Mg^{2+}), subpackage the solution and store at -25℃ ~ -15℃ (The reagent can be incubated at 37℃ for 3 ~ 5 min to help dissolve the powder). Avoid repeated freezing, thawing and shaking. The enzyme solution can be stored stably for 6 months at -25℃ ~ -15℃.

(2) Preparation of enzyme B solution: Dissolve the powder of the enzyme B reagent with 1.4 mL buffer B, subpackage the solution and store at -25℃ ~ -15℃. Avoid repeated freezing, thawing and shaking. The enzyme solution can be stored stably for 6 months at -25℃ ~ -15℃.

(3) Preparation of enzyme C solution: Dissolve the powder of the enzyme C reagent with 2.7 mL buffer C, subpackage the solution and store at -25℃ ~ -15℃. Avoid repeated freezing, thawing and shaking. The enzyme solution can be stored stably for 6 months at -25℃ ~ -15℃.

(4) Preparation of enzyme mixture: Prepare the mixture according to the table below, and the enzyme mixture is freshly prepared just before use. The enzyme mixture can be used to process 20 ~500 mg muscle tissue. When processing tissue greater than 500 mg, the amount of tissue processing tube needs to be increased. If subsequent cell culture is necessary, the mixture needs to be sterile-filtered through the 0.22 μm syringe filter and make sure the total volume of filtered mixture is 2 mL.

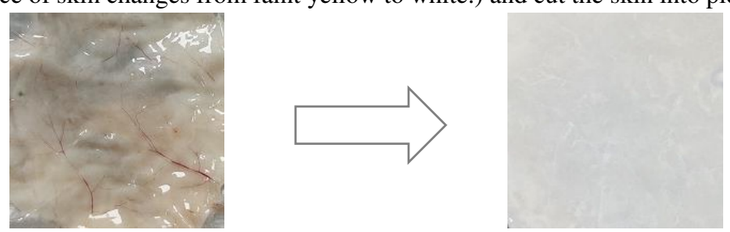
Tissue Type	Weight Range	Enzyme Mixture
Back Skin	100 ~ 500 mg	1.75 mL Buffer D + 100 μL Enzyme A + 50 μL Enzyme B + 100 μL Enzyme C
Ear Skin	20 ~ 300 mg	

⚠ Note: Enzyme A solution should be fully dissolved in the 37℃ constant temperature oscillator before use.

Mechanized Protocol

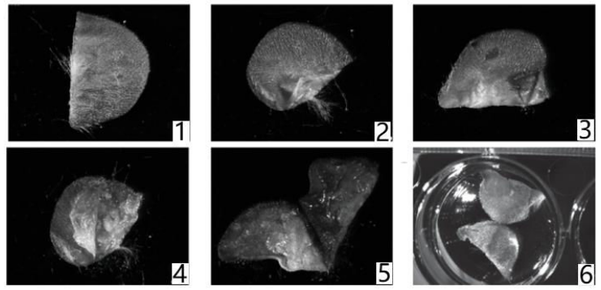
(1) Implement euthanasia by cervical dislocation on the mouse aged 6 ~ 10 weeks and use the hair removal device to remove the hair of back skin. Then, apply the hair removal cream on the back skin and wait for 3 ~ 5 min before cleaning the cream. Cut off the processed skin and place it in the cold PBS buffer.

(2) Back skin: Gently separate the skin peritoneum from muscle. Hold the skin sample by forceps while separating the peritoneum from muscle with a surgical scissors or scalpel, wash the sample with cold PBS buffer 3 times until no impurity is found, (It is demonstrated that the skin is processed well when the color of the inner surface of skin changes from faint yellow to white.) and cut the skin into pieces of 2 ~ 4 mm.



(Before〉 (After)

Ear skin: Cut off the hairless part of the mouse ear and separate the tergal side from the ventral side with a forceps, scrape the ear skin with the forceps to remove the cartilage and cut the skin into pieces of 2 ~ 4 mm pieces.



(3) Weigh the skin tissue according to the weight range. Transfer the tissue pieces to the tissue processing tube containing enzyme mixture, tighten then tube cap and blend the mixture and tissue well.

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

(4) Invert the tissue processing tube, mount it in the bushing of the single cell suspension dissociator with the heater and run the program **M_Skin_Heater_1**.

(5) After the program is finished, remove the tissue processing tube from the single cell suspension dissociator. Wet the 40 μm cell strainer with 1 mL RPMI 1640 or DMEM medium, filter the cell suspension through the cell strainer, and collect the cell suspension to a 50 mL centrifuge tube.

(6) Rinse the tissue processing tube with 10 mL RPMI 1640 or DMEM medium, filter the suspension through the 40 μm cell strainer and collect it to the 50 mL centrifuge tube in step (5). Transfer the suspension to a 15 mL centrifuge tube.

(7) Centrifugate the cell suspension at 500×g for 8 min at room temperature 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.

⚠ Note: If the suspension still has floc, it is suggested to filter the suspension through the 40 μm cell strainer.

(Optional) Removal of floc can be performed with the help of the attached high efficiency debris removal reagent.

Tissue Weight	PBS Volume (For Resuspension)	High Efficiency Debris Removal Reagent Volume	Upper PBS Volume	Applicable Tube
200 ~ 500 mg	1.55 mL	450 μL	2 mL	15 mL Centrifuge Tube

⚠ Note: Sample weighing less than 200 mg do not require floc removal.

① According to the table above, resuspend the cell precipitate with 1.55 mL pre-cooled PBS buffer, add 450 μL high efficiency debris removal reagent and use a 1 mL pipette to gently blow and blend the suspension 5 ~ 10 times (The bloc should be blown apart). Then, slowly drip 2 mL pre-cooled PBS buffer along the tube wall and tighten the tube cap.

② Slowly place the centrifuge tube in the centrifuge, 3000×g, 4℃, acceleration 9 and deceleration 3 for 10 min. After the centrifugation is finished, use the pipette to discard the supernatant.

③ Resuspend the suspension with RPMI 1640 or DMEM medium to required volume for subsequent experiment.

⚠ Note: In step (2), please place or remove the tube as slowly as possible and keep the tube vertical, avoiding shaking that may cause the floc to disperse.

Manual Protocol

(1) Follow the steps (1) ~ (3) in ***"Mechanized Protocol"*** to prepare the tissue and place the tissue processing tube containing tissue and enzyme in the 37℃ constant temperature oscillator for 1 h.

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

(2) Wet the 40 μm cell strainer with 1 mL RPMI 1640 or DMEM medium, filter the suspension through the cell strainer. Rinse the tissue processing tube or the centrifuge tube with 5 mL RPMI 1640 or DMEM medium, filter through the 40 μm cell strainer and collect all the suspension to the 50 mL centrifuge tube.

(3) Transfer the 40 μm cell strainer to the 60 mm petri dish, add 3 mL RPMI 1640 or DMEM medium to immerse the tissue and gently grind the tissue by syringe piston (end) for 30 s. It can be observed that the cells flow out and the solution becomes turbid. Filter the solution in the petri dish through the 40 μm cell strainer.

(4) Rinse the petri dish with 5 mL RPMI 1640 or DMEM medium, filter the solution through the 40 μm cell strainer and collect it to the 50 mL centrifuge tube in step (2). Then, transfer the suspension to the 15 mL centrifuge tube.

(5) Centrifugate the suspension at 500×g for 8 min at room temperature and discard the supernatant.

(6) Resuspend the suspension with RPMI 1640 or DMEM medium to required volume for subsequent experiment.

⚠ 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) When processing the tissue, it is suggested to remove as much fat and muscle tissue as possible to avoid affecting subsequent experiment.

(4) The mouse aged 6 ~ 10 weeks can help get ideal experiment results, while the mouse aged over 10 weeks may influenced the results.

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

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