High Activity Whole Skin EnzymaticDigestion Kit (Mouse) Instructions

1 Product Information

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

2 Description

High Activity Whole Skin Enzymatic Digestion Kit (Mouse) can prepare the skin tissue on the back or ear of an adult mouse (6 ~ 10 weeks) into single cell suspension in a gentle, quick, and efficient manner. This optimization scheme can help obtain as many highly-viable single cell samples as possible while maintaining the important surface epitopes of cells. The obtained single cell suspension can continue to be applied in downstream experiments such as cell sorting and primary cell culture.

Main principle: Mouse skin tissue is prepared as a single cell suspension by a combination of mechanical shearing and enzymatic digestions of the extracellular matrix to keep the tissue structurally intact. The Single Cell Suspension Dissociator (RWD) is chiefly used for mechanical dissociation, while the High Activity Whole Skin Enzymatic Digestion Kit (Mouse) mainly digests the tissue through enzymatic hydrolysis. After dissociation, the sample is filtered using a cell strainer to remove tissue residues to obtain a single cell suspension, and the obtained cells can be used immediately for subsequent experiments, such as primary cell culture, cell sorting, single cell sequencing.

3 Components

7 bottles of reagents in total, including:

1 bottle of Enzyme A (powder)
1 bottle of Enzyme B (powder)
1 bottle of Enzyme C (powder)
1 bottle of Buffer B (solution)
1 bottle of Buffer C (solution)
1 bottle of Buffer D (solution)
1 bottle of High efficiency debris removal Kit (solution)

4 Test Capacity

The kit can dissociate the mouse skin (dorsal or ears) tissue 25 times, processing 20 mg ~ 500 mg of the tissue each time.

5 Transport and Storage

Ship the kit with an ice pack at 2 ~ 8℃. Store the Enzyme C powder in a refrigerator at -25 ~ -15℃, with the Enzyme A and B powder and buffer solutions (B, C and D) and High efficiency debris removal Kit stored in a refrigerator at 2 ~ 8℃. The shelf life of all is 12 months.

6 Reagent and Instrument Requirements

DSC-400/DSC-800 single cell suspension dissociator (RWD);
HJ-400 Heater (RWD);
Tissue processing tube* (RWD);
Hanks (containing Ca^{2+}, Mg^{2+} and Phenol Red)(Solarbio: #H1020);
Constant temperature oscillator;
PBS ;
40 μm cell strainer.

7 Method for Use

7.1 Reagent Preparation

1) Enzyme A solution: dissolve the Enzyme A powder thoroughly in 2.7 mL Hanks(containing Ca^{2+}, Mg^{2+} and Phenol Red);
2) Enzyme B solution: dissolve the Enzyme B powder thoroughly in 1.4 mL Buffer B;
3) Enzyme C solution: dissolve the Enzyme C powder thoroughly in 2.7 mL Buffer C.

To avoid repeated freezing and thawing, prepare a proper amount of EP tubes to contain all components of dissolved enzyme solutions separately (Enzyme A powder must to be completely dissolved before packaging, can use 37°C Constant temperature oscillator to accelerate the dissolution), and cryopreserve them at -25℃ to -15℃, with validity period of 6 months. If subsequent cell culture is required, the enzyme mixture should be sterile-filtered with a 0.22 μm syringe filter. After filtration, the total volume of enzyme mixture should be 2 mL.

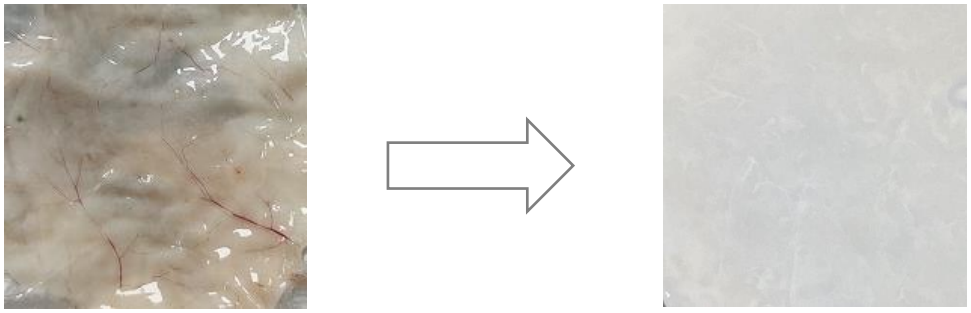
Tissue Types	Sample Range	Enzyme mixture
Dorsal skin	100 mg ~ 500 mg	1.75 mL Buffer D + Enzyme A 100 μL+ Enzyme B 50 μL + Enzyme C 100 μL
Skin on ears	20 mg ~ 300 mg	1.75 mL Buffer D + Enzyme A 100 μL+ Enzyme B 50 μL + Enzyme C 100 μL

⚠ Note: Enzyme A solution should be fully dissolved in a 37°C Constant temperature oscillator before use.

7.2 Solutions for High Activity Skin Tissue Enzymatic Digestion

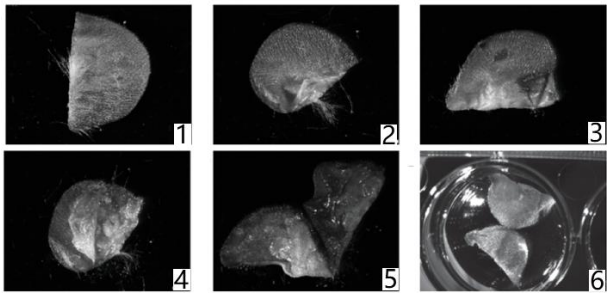
7.2.1 Application of DSC-400/DSC-800 Single Cell Suspension Dissociator (HJ-400 Heater Included)

1) Take an ICR or Balb/c mouse that is 6 ~ 10 weeks old, and euthanize it by performing dislocation of cervical vertebrae. After using a hair removal device to depilate the dorsal skin, apply the hair removal cream on the dorsal skin of the mouse. The hair removal cream can be removed once standing for 3 ~ 5 minutes. Cut off the depilated skin tissue and place it into cold PBS.
2) Dorsal skin: Gently dissect the skin peritoneum from the dorsal muscle. Keep the skin sample still with forceps while dissecting the skin sample by using the closed, rounded tip of a pair of surgical scissors or a scalpel. Wash the skin sample 3 times with cold PBS until no impurities are present (if the color of the inner skin can be observed to turn from light yellow to white, it indicates that the skin is washed clean), then cut the skin into little pieces with each about 2 ~ 4 mm long.



(The dorsal skin tissue before treatment) (The dorsal skin tissue after treatment)

Skin on ears: Cut off the hairless parts of the mouse's ears, separate the dorsal and ventral sides with forceps, and scrape the inner sides of the parts to remove the remaining cartilage, then cut the skin into little pieces with each about 2 ~ 4 mm long.



3) Weigh the tissue according to the sample range. Transfer the tissue blocks to a tissue processing tube containing enzyme mixture. Tightening the cover of the tissue processing tube and mix the contents gently.
   ⚠ Note: Make sure the sample material is in the area where the rotor/stator is located.
4) Insert the tube upside down into a sleeve of the DSC-400/DSC-800 Single Cell Suspension Dissociator, install the Heater, and run the program "**M_Skin_Heater_1**".
5) When the program runs to the end, continue with Step 5) in **7.2.2** until the end of the experiments.

7.2.2 Application of DSC-400/DSC-800 Single Cell Suspension Dissociator Alone

1) Pretreat the skin tissue as per Steps 1) and 2) in **7.2.1**;
2) Weigh the tissue according to the sample range. Transfer the tissue blocks to a tissue processing tube containing enzyme mixture. Tightening the cover of the tissue processing tube and mix the contents gently.
   ⚠ Note: Make sure the sample material is in the area where the rotor/stator is located.
3) When the constant temperature oscillator remains at 37℃, and the rotational speed is set to “0”, place the tube upside down into it and incubate the tube in the water bath for 60 minutes.
4) After incubation, insert the tube upside down into DSC-400/DSC-800 Single Cell Suspension Dissociator, and run the program **"Mouse_Skin_1"**.
5) At the end of the program, remove the tissue processing tube from the single cell suspension dissociator, rinse the 40 μm cell strainer with 1 mL PBS, filter the cell suspension sample using the wetted cell strainer, and collect the cell suspension into a 50 ml centrifuge tube.
6) Then wash the tissue processing tube with 9 mL PBS. Filter the resulting solution with the 40 μm strainer and collect it into the 50 mL centrifuge tube mentioned in Step 5). Transfer the cell suspension from the 50 mL centrifuge tube to a 15 mL centrifuge tube.
7) Centrifuge the cell suspension for 8 minutes at 500×g and at room temperature. Completely discard the supernate.
8) Resuspend the cells to the desired volume with an appropriate amount of PBS for further applications.
   ⚠ Note: If floccules remain in the obtained single cell suspension, you can filter it again with the 40 μm cell strainer.
9) Optional) To remove flocs from cell suspension, use the High efficiency debris removal Kit in the kit to remove flocs.

Weight of Tissue	Cell Volume of PBS Resuspension	Volume of High efficiency debris removal Kit	Volume of Upper PBS	Applicable pipe
200 ~ 500 mg	1.55 mL	450 μL	2 mL	15 mL tube

⚠ Note: No flocculation treatment is required for samples weighing less than 200 mg.

① According to the above table, 1.55 mL of pre-cooled PBS was used to re-suspend cell precipitate, adding 450 μL of the High efficiency debris removal Kit, and then gently blown with a 1 mL pipette gun for 5 ~ 10 times mixing (the flocculent should be blown away), then 2 mL of pre-cooled PBS was slowly dropped along the wall of the centrifuge tube, and tightening the bottle cap

② Slowly put the cell suspension from the step ① into the centrifuge, setting the centrifugal parameters: 3000 ×g, 4℃, acceleration 9, deceleration 3, centrifuge for 10 min. After centrifugation, remove the centrifuge tube and use the pipette to completely discard the supernatant.

③ The cells were suspended to the required volume with PBS for subsequent experiments.
   ⚠ Note: In step ②, put in or take out the centrifugal tube as slowly as possible and keep the tube in a vertical state to avoid shaking causing flocculent dispersion.

8 Precautions

1) The shelf life of this kit is 12 months, and RWD will not guarantee the validity of expired products.
2) When downstream cell culture is carried out after tissue dissociation, make sure that all operations are performed under sterile conditions.
3) Unnecessary fat and muscle tissue should be removed from the skin as much as possible during tissue pretreatment, thus reducing the influence of impurities on the following experiments.
4) It is better to control the age of experimental mouse within 6 ~ 10 Weeks, and the experimental effect will be different after 10 weeks.

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

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