High Activity Adipose Tissue Enzymatic Digestion Kit (Mouse & Rat) Instructions

Product Information

Product Name	Model	Specification
High Activity Adipose Tissue Enzymatic Digestion Kit (Mouse & Rat)	DHAE-5010	50 T

Product Description

High Activity Adipose Tissue Enzymatic Digestion Kit (Mouse & Rat) (the "Kit") can prepare adipose tissue of mouse and rat 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, which is immature adipose cell suspension, can be applied in downstream experiments such as SVF cell culture, cell sorting 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 the adipose 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 Adipose Tissue Enzymatic Digestion Kit (Mouse & Rat)	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
White Adipose Tissue	66 T	100 ~ 1000 mg to be processed per time
Beige / Brown Adipose Tissue	50 T	50 ~ 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	HBSS Buffer (with $Ca^{2+}$and Mg^{2+})	PBS	RPMI 1640 or DMEM Medium
	Red Blood Cell Lysis Buffer (optional)		
Consumable	Tissue Processing Tube (RWD)	Heater (RWD: # HJ-400)	100 μm Cell Strainer
	40 μm Cell Strainer	0.22 μm Syringe Filter (optional)	
Instrument	Single Cell Suspension Dissociator (RWD)	Constant Temperature Oscillator	Vortex Oscillator

Operation

Preparation

(1) Preparation of enzyme A solution: Dissolve the powder of the enzyme A reagent with 6 mL HBSS Buffer (with $Ca^{2+}$and Mg^{2+}) in the 37℃ constant temperature oscillator, subpackage 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 mL buffer B, subpackage 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: Dissolve the powder of the enzyme C reagent with 1.5 mL buffer C, subpackage 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 according to the table below, and the enzyme mixture should be freshly prepared just before use. The enzyme mixture can be used for processing 0.1 ~ 1.0 g white adipose tissue and 0.05 ~ 0.5 g beige or brown adipose tissue. When processing adipose tissue greater than the above range, the amount of tissue processing tube should be increased. If subsequent cell culture is required, the enzyme mixture 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.

Tissue Type	Enzyme Mixture			
White Adipose Tissue	HBSS Buffer (with $Ca^{2+}$and Mg^{2+}) 2.4 mL	Enzyme A 75 μL	Enzyme B 75 μL	Enzyme C 12.5 μL
Beige / Brown Adipose Tissue	HBSS Buffer (with $Ca^{2+}$and Mg^{2+}) 2.3 mL	Enzyme A 100 μL	Enzyme B 100 μL	Enzyme C 25 μL

⚠ Note: HBSS buffer (with $Ca^{2+}$and Mg^{2+}) can be temporarily replaced by RPMI 1640 medium.

Mechanized Protocol

(1) Prepare the enzyme mixture in the tissue processing tube following in ***"Preparation"***.

(2) After obtaining adipose tissue, rinse the tissue repeatedly with PBS and put it in the petri dish containing RPMI 1640 or DMEM medium for temporary storage. Cut the tissue into pieces of 2 ~ 4 mm and weigh target weight of tissue pieces with the electronic balance scale.
⚠ Note: The adipose tissue should be freshly prepared and used. Remove the lymph gland, blood and connective tissue diaphragm as much as possible. Pay attention to maintaining the stromal vascular fraction.

(3) Transfer the tissue pieces to the tissue processing tube containing enzyme mixture, 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 white adipose tissue, run the program **M_Adipose_Heater_1**; for beige or brown adipose tissue, run the program **M_Adipose_Heater_2**.

(5) After the program is finished, remove the tissue processing tube from the single cell suspension dissociator.

(6) Wet the 100 μm cell strainer with 1 mL RPMI 1640 or DMEM medium, filter the cell suspension through

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the wetted cell strainer, and collect the cell suspension to the 50 mL centrifuge tube.

(7) 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 (6).

⚠ Note: For denser adipose tissue, if more residue is found after filtering, the undigested tissue pieces can be blown 3 ~ 5 times with the pipette aspirated with RPMI 1640 or DMEM medium to help release more single cells, but the viability of the cell suspension will be lower.

(8) When processing small amount of tissue, the amount of cells obtained may be low. To reduce the loss of cells, it is suggested to collect all the cell suspension at 15 mL centrifuge tube and perform centrifugation at 300×g for 10 min. After the centrifugation, discard the supernatant completely.

⚠ Note: To avoid loss of SVF cell pellet, please do not pour the suspension directly.

(Optional) Removal of red blood cells

If it is necessary to remove the red blood cells, resuspend the cells collected in step (8) with 0.5 ~ 1 mL red blood cell lysis buffer. Then, incubate the suspension on ice for about 2 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.

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

⚠ Note: For floc in the cell suspension, it is suggested to gently blow the suspension and then filter the suspension through 40 μm cell strainer.

Manual Protocol

(1) Prepare the enzyme mixture in the 50 mL centrifuge tube following in ***"Preparation"***.

⚠ Note: HBSS buffer (with $Ca^{2+}$and Mg^{2+}) can be temporarily replaced by RPMI 1640 medium.

(2) After obtaining adipose tissue, rinse the tissue repeatedly with PBS and put it in the petri dish containing RPMI 1640 or DMEM medium for temporary storage. Cut the tissue into pieces of 2 ~ 4 mm and weigh target weight of tissue pieces with the electronic balance scale.

⚠ Note: The adipose tissue should be freshly used and remove as much lymph gland, blood and connective tissue diaphragm. Pay attention to maintaining the stromal vascular fraction.

(3) Transfer the tissue pieces to the 50 mL centrifuge tube containing enzyme mixture, place the tube in the 37°C constant temperature oscillator and incubate the cell suspension.

(4) The white adipose tissue should be oscillated in the vortex oscillator for 10 ~ 15 s after the incubation for 10 min. Blend the tissue and enzyme mixture, place the tube in the 37°C constant temperature oscillator and incubate the suspension again. The time of incubation should not exceed 40 min. During the incubation, oscillate the suspension 2 ~ 3 times and the tissue of small initial dosage can be basically digested. For the tissue sample of large amount, the tissue residue can be blown by the l mL pipette with wide-nosed tips or the 1 mL pointed-tip pipette, which the tip is cut off 0.5 cm; for beige or brown tissue, it should be incubated in the 37°C constant temperature oscillator at 60 rpm, and be oscillated in the vortex oscillator for 10 ~ 15 s each 10 min. The time of incubation should be in the range of 40 ~ 50 min. During the digestion, it is suggested to observe carefully the condition to avoid over-digestion.

(5) After the digestion, add 10 mL RPMI 1640 or DMEM medium to stop the digestion.

(6) Follow the steps in ***"Mechanized Protocol"*** from step (6) to (9). For residue on the cell strainer, it is suggested to blow and grind the residue with the l mL pipette aspirated with appropriate volume of medium.

⚠ 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) White adipose tissue (eWAT) is visceral fat, mostly taken from the testes and epididymis and other gonads in the abdominal cavity, so please pay attention when removing other tissues adhered to and impurities; brown adipose tissue (BAT) is mostly taken from the middle of the scapula in the subcutaneous area, covered by beige adipose tissue, so please pay attention when removing the beige adipose and the thin membrane in the middle of the beige adipose; beige adipose tissue (iWAT) is subcutaneous adipose tissue, mostly taken from the groin, so please pay attention when removing lymph nodes in the beige adipose and the surrounding membrane; clean adipose tissue can avoid the interference of other cells that in the pre-processing, the adipose tissue should be soaked in buffer or medium as much as possible, with gentle and rapid operation.

(4) SVF cell is stromal vascular component cell, and the entangled blood vessels in the adipose tissue are the main source of target cells, so please pay attention to preserving as many stromal vessels as possible when obtaining and processing the adipose tissue.

(5) It is suggested to process the tissue according to the recommended tissue weight to avoid excessive residue and inadequate digestion.

(6) 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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