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

Product Information

Product Name	Model	Specification
High Activity Muscle Tissue Enzymatic Digestion Kit (Mouse & Rat)	DHME-5012	50 T

Product Description

High Activity Muscle Tissue Enzymatic Digestion Kit (Mouse & Rat) (the "Kit") can prepare muscle tissue of adult or newborn 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 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 muscle 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 Muscle Tissue Enzymatic Digestion Kit (Mouse & Rat)	Enzyme A Reagent (powder)	1 vial	-25℃ ~ -15℃
	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 & Rat Muscle Tissue	50 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	PBS Buffer	Red Blood Cell Lysis Buffer (optional)
	High Efficiency Debris Removal Kit (optional, RWD: # DHDR-5006)		
Consumable	Tissue Processing Tube (RWD)	Heater (RWD # HJ-400)	70 μm Cell Strainer
	0.22 μm Syringe Filter (optional)		
Instrument	High-Speed Benchtop Refrigerated Centrifuge (RWD: # M1416R)	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 7.5 mL DMEM, 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℃.

(2) Preparation of enzyme B solution: Dissolve the powder of the enzyme B reagent with 7.5 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 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 should be freshly prepared just before use. The enzyme mixture can be used for processing up to 500 mg muscle tissue. When processing muscle tissue greater than 500 mg, the amount of tissue processing tube should be increased.

Enzyme Mixture			
DMEM 1660 μL	Enzyme A 150 μL	Enzyme B 150 μL	Enzyme C 40 μL

Mechanized Protocol

(1) After obtaining muscle tissue of adult or newborn mouse and rat, put it in the petri dish containing DMEM medium or PBS buffer, strip off the achilles tendon and cut the tissue into pieces of 2 ~ 4 mm.

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

(3) Tighten the tissue processing tube, invert it and mount it in the bushing of the single cell suspension dissociator with the heater. Run the program **M_Muscle_Heater_1**.

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

(4) After the program is finished, remove the tissue processing tube from the single cell suspension dissociator. Centrifugate the tube instantly for 3 s or at 300×g for 5 s to sink the tissue at the bottom of the tube. Use a 1 mL pipette which the tip is cut off about 5 mm to blow and blend the suspension 10 ~ 15 times.

(5) Wet the 70 μm cell strainer with 1 mL DMEM medium, filter the cell suspension through the cell strainer, and collect the cell suspension to the 50 mL centrifuge tube.

(6) Rinse the tissue processing tube with 10 mL DMEM medium, filter the suspension through the 70 μm cell strainer and collect it to the 15 mL centrifuge tube.

(7) Centrifugate the cell suspension at 300×g for 20 min and discard the supernatant completely.

(Optional) Removal of cell debris

If observed that the suspension has much debris, it is recommended to use the High Efficiency Debris Removal Kit (RWD: # DHDR-5006) to remove the debris. This process will cause the loss of cells that the amount of the cells should be greater than 1×10^6 when using the debris removal kit.

⚠ Note: When the amount of the cells are less than 1×10^6, the debris layer may not be visible.

(Optional) Removal of red blood cells

If it is necessary to remove the red blood cells, resuspend the cells collected in step (7) with 500 μL red blood cell lysis buffer. Then, incubate the suspension on ice for 2 ~ 3 min, followed by resuspension with

9 mL DMEM medium. Centrifugate the cell suspension at 300×g for 10 min and discard the supernatant completely. Resuspend the cell suspension to the desired volume with appropriate medium or buffer for subsequent experiment.

⚠ Note: When the cell debris and the red blood cells are both need to be removed, please conduct the removal of cell debris first.

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

Manual Protocol

(1) After obtaining muscle tissue of adult or newborn mouse and rat, put it in the petri dish containing DMEM medium or PBS buffer, strip off the achilles tendon and cut the tissue into pieces of 2 ~ 4 mm.

(2) Transfer the tissue pieces to the tissue processing tube or centrifuge tube containing enzyme mixture and tighten the cap.

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

(3) Incubate the cell suspension in the 37°C constant temperature oscillator at 50 rpm for 20 min and then oscillate it in the vortex oscillator at intermediate speed for 20 s.

(4) Repeat step (3) once again.

(5) Incubate the cell suspension in the 37°C constant temperature oscillator at 50 rpm for 20 min and then use the 1 mL pipette which the tip is cut off about 5 mm to blow and blend the suspension 20 times.

(6) 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.

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

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