High Activity Muscle Tissue Enzymatic Digestion Kit (Mouse and Rat)

1 Product Information

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

2 Description

The High Activity Muscle Tissue Enzymatic Digestion Kit (Mouse and Rat) allows for the gentle, rapid and efficient dissociation of mouse implanted muscle tissues into single cell suspensions. This optimized protocol allows for the highest possible number of single cell samples with high cell viability while preserving important surface antigenic epitopes of the cells. The single cell suspension obtained can continue to be used for downstream experiments of muscle cells such as cell culture or cell sorting etc.

Principle: Muscle tissues are dissociated into single-cell suspensions by a combination of mechanical dissociation and enzymatic digestion of the extracellular matrix (to maintain structural integrity of the tissue). RWD single cell suspension dissociator mainly plays the role of mechanical dissociation, while the High Activity Muscle Tissue Enzymatic Digestion Kit (Mouse and Rat) mainly digests the tissue by enzymatic digestion. After dissociation, the sample is filtered with a cell filter to remove tissue residues in the sample to obtain a single-cell suspension, and the cells obtained can be used immediately for subsequent experiments, such as cell isolation, cell culture etc.

3 Components

5 vials of reagents, including:

1 vial of Enzyme A reagent (lyophilized powder)
1 vial of Enzyme B reagent (lyophilized powder)
1 vial of Enzyme C reagent (lyophilized powder)
1 vial of Buffer B
1 vial of Buffer C

4 Capacity

50 muscle tissue digestions, with 20 ~ 500 mg of muscle tissue digested each time.

5 Transport and Storage

Transport at -25 ~ -15°C;

All components are stored at -25 ~ -15°C, with validity period of 12 months.

6 Reagent and Instrument Requirements

PBS

DMEM

Red blood cell lysis buffer (Optional)

High efficiency debris removal Kit (Optional，RWD: # DHDR-5006)

70 μm cell strainer

Heater (RWD: # HJ-400)

Constant temperature oscillator

Tissue processing tube* (RWD)

Single cell suspension dissociator (RWD)

7 Method for Use

7.1 Reagent preparation

7.1.1 Dissolve enzyme dry powder

(1) Prepare Enzyme A solution: dissolve the powder in the Enzyme A reagent bottle with 7.5 mL DMEM. After dissolution, sub-pack the solution directly, followed by frozen storage at -25 ~ -15°C to avoid

repeated freezing and thawing and vibrate agitation. The enzyme solution can be stored stably for 6 months at -25 ~ -15℃.

(2) Prepare Enzyme B solution: dissolve the powder in the Enzyme B reagent bottle with 7.5 mL Buffer B. After dissolution, sub-pack the solution directly, followed by frozen storage at -25 ~ -15°C to avoid repeated freezing and thawing and vibrate agitation. The enzyme solution can be stored stably for 6 months at -25 ~ -15℃.

(3) Prepare Enzyme C solution: dissolve the powder in the Enzyme C reagent bottle with 2 mL Buffer C. After dissolution, sub-pack the solution directly, followed by frozen storage at -25 ~ -15°C to avoid repeated freezing and thawing and vibrate agitation. The enzyme solution can be stored stably for 6 months at -25 ~ -15℃.

7.1.2 Prepare enzyme mixture

Prepare mix 1 according to the table below, and the enzyme mixture is freshly prepared just before use. The Enzyme mix 1 prepared below can be used for up to 500 mg muscle tissue. If the weight of muscle tissue treated is greater than 500 mg, the number of tissue processing tubes needs to be increased.

Enzyme mix 1			
DMEM 1660 µL	Enzyme A 150 µL	Enzyme B 150 µL	Enzyme C 40 µL

7.2 Protocol for Muscle Tissue Gentle Enzymatic Digestion

(1) After stripping the muscle tissue from adult or neonatal mouse&rat, place and temporarily store the tissue in a petri dish containing DMEM or PBS at 4°C with solution overhead the muscle tissue, then cut it into small pieces of 2 ~ 4 mm size, and remove Achilles tendon gently from the above tissue as much as possible.

(2) Transfer the muscle tissue to the tissue processing tube included with Enzyme mix 1.

(3) Tighten the tissue processing tube, turn it upside down, and fit into the channel of single cell suspension dissociator and run **M_Muscle_Heater_1**.

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

(4) After the program ends, remove the tissue processing tube from the single cell suspension dissociator to centrifuge, and short for 3s or centrifuge at 300×g for 5s to sink the sample tissue to the tube bottom. Using a 1 mL pipette, blow the mixed cell suspension 10 ~ 15 times.

(5) Wet a 70 µm cell strainer with 1 mL of DMEM, and filter the cell suspension sample with the wetted cell strainer, collect the cell suspension in a 50 mL centrifuge tube.

(6) Rinse the tissue processing tube with 10 mL DMEM, after filtering through a 70 µm filter, collect it in a 15 mL centrifuge tube.

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

(Optional) Debris removal

If you want to remove flocs from cell suspension, use the high efficiency debris removal kit (RWD: # DHDR-5006) to remove debris. However, the debris removal will lose some cells, and it is recommended that the number of cells is greater than 1×10^6 when the debris is removed.

⚠ Note: When debris is removed, the number of cells is less than 1×10^6, there are fewer debris, and the debris layer may not be visible.

(Optional) Red blood cells removal

If erythrocyte removal is required, resuspend the cells treated in step (7) with 500 µL of 1× erythrocyte lysis solution, then incubate on ice for 2 ~ 3 min, followed resuspend by 9 mL of DMEM, centrifuge the cell suspension at 300×g for 10 minutes, completely discard the supernatant, and resuspend the cells in the appropriate buffer or medium by pipetting slowly up and down for subsequent experiments.

⚠ Note: When debris and red blood cell removal are required at the same time, debris removal is performed first.

(8) Resuspend cells to a desired volume with DEME or appropriate buffer for follow-up experiments.

8 Precautions

(1) This kit is valid for 12 months, and RWD shall 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.

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

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