High Activity Intestine Tissue Enzymatic Digestion Kit (Mouse) Instructions

Product Information

Product Name	Model	Specification
High Activity Intestine Tissue Enzymatic Digestion Kit (Mouse)	DHIE-5007	50 T

Description

The High Activity Intestine Tissue Enzymatic Digestion Kit (Mouse) can prepare mouse (6 ~ 10 weeks) intestine (the small intestine) tissue into single cell suspension gently, quickly and efficiently. This optimized protocol can help obtain as many highly-viable single cell suspension 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 intestine 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	Specification	Storage Condition
High Activity Intestine Tissue 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	-25℃ ~ -15℃
	Buffer E (solution)	1 vial	-25℃ ~ -15℃
	Buffer H (solution)	1 vial	2℃ ~ 8℃

Test Capacity

Recommendation for single process of tissue:

Tissue Type	Capacity	Initial Sample Dosage
Mouse Small Intestine Tissue	50 T	200 ~ 600 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+})	HBSS Buffer (without $Ca^{2+}$and Mg^{2+})	Red Blood Cell Lysis Buffer (optional)
	FBS (Fetal Bovine Serum)	PBS Buffer	RPMI 1640 or DMEM Medium
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 5.5 mL HBSS buffer (with Ca^{2+} and Mg^{2+}), 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 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 1.4 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) Buffer D and buffer E can be directly subpackaged and stored at -25℃ ~ -15℃. Avoid repeated freezing and thawing. Buffer H should be stored at 2℃ ~ 8℃ after dilution.

(5) Preparation of cleaning solution:

Volume	Preparation	PH
20 mL	17.8 mL HBSS buffer (without $Ca^{2+}$and Mg^{2+}) + 1 mL FBS + 20 μL Buffer D + 1 mL Buffer E + 200 μL Buffer H	Add 25 μL 4M NaOH to adjust the PH value to 7.2 ~ 7.4

(6) 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 to process 200 mg ~ 600 mg intestine tissue. When processing intestine tissue greater than 600 mg, the amount of tissue processing tube should be increased. If subsequent cell culture is required, the enzyme mixture should be sterile-filtered through the 0.22 μm syringe filter and the volume of the filtered mixture should be 2.5 mL in total.

Range	Enzyme Mixture			
200 mg ~ 600 mg	2.12 mL HBSS buffer (with $Ca^{2+}$and Mg^{2+}) + 20 μL Buffer H + 210 μL FBS	Enzyme A 100 μL	Enzyme B 25 μL	Enzyme C 25 μL

Mechanized Protocol

(1) Place the intestine (mainly the small intestine) of the mouse aged 6 ~ 10 weeks in the petri dish containing cold PBS buffer.

(2) Remove redundant fat, lymphatic tissue and blood by surgical device. Then, incise the intestine tissue longitudinally, clean the excrement and wash the intestine with cold PBS buffer 3 ~ 5 times until no obvious impurities. Scrub the surface water of the intestine by surgical device and cut the intestine transversely into tissue pieces of 2~ 4 mm.

⚠ Note: Cut the tissue as small as possible, otherwise large tissue pieces will clog in the tissue processing tube.

(3) Weigh the tissue according to the range and transfer the tissue to the 50 mL centrifuge tube containing 20 mL PBS buffer. Place the centrifuge tube on the vortex oscillator at 1000 ~ 1500 rpm for 30 s (to shake the tissue as much as possible) and filter out the PBS buffer through the 100 μL cell strainer.

(4) Transfer the tissue pieces on the cell strainer to the 50 mL centrifuge tube containing 20 mL cleaning solution and place the tube in the 37℃ constant temperature oscillator at 150 rpm for 30 min. It is noted that the tube should be placed aslant for fully shaking of tissue.

⚠ Note: If there is no constant temperature oscillator, constant temperature shaker can be the alternative.

(5) After cleaning, filter the tissue through the 100 μm cell strainer and transfer the tissue pieces to the 50 mL centrifuge tube containing 20 mL PBS buffer. Wash 10 times upside down (to shake the tissue as much as possible) and again filter through the 100 μm cell strainer to discard the PBS buffer.

(6) Repeat the step (5) once again.

(7) Transfer the tissue to the tissue processing tube containing enzyme mixture, tighten the tube and shake it slightly for blending.

(8) Invert the tissue processing tube and mount it in the bushing of the single cell suspension dissociator with heater.

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

(9) Run the program **M_Intestine_Heater_1**.

(10) After the program is finished, remove the tissue processing tube from the single cell suspension dissociator. Wet the 100 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 the 50 mL centrifuge tube.

(11) 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 (10).

⚠ Note: Filter the suspension again through the 40 μm cell strainer.

(12) Centrifuge the cell suspension at 300×g for 10 min at room temperature and completely discard the supernatant.

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

Manual Protocol

(1) Follow the steps (1) ~ (6) in ***"Mechanized Protocol"***. Then, add the tissue pieces to the 50 mL centrifuge tube containing enzyme mixture. Tighten the tube cap and place the tube in the 37℃ constant temperature oscillator at 50 rpm for 30 min for incubation.

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

(2) After incubation is finished, take out the tissue processing tube or the centrifuge tube and add 3 mL RPMI 1640 or DMEM medium to end the digestion. Then, use a 1 mL pipette to blow the tissue suspension until no obvious tissue pieces exist (The tip of pipette should be cut off about 0.5 cm).

(3) Rinse the 100 μm cell strainer with 1 mL RPMI 1640 or DMEM medium and filter the cell suspension through the cell strainer. Wash the tissue processing tube or the centrifuge tube with 100 mL RPMI 1640 or DMEM medium again, filter the suspension through the 100 μm cell strainer and collect the suspension to the 50 mL centrifuge tube.

(4) Follow the steps (12) ~ (13) in ***"Mechanized Protocol"***.

⚠ 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) Steps (5) and (6) in ***"Mechanized Protocol"*** have a great impact on the results, so these two steps can not be omitted.

(4) Buffer D has low toxicity, please wear protective gloves to avoid contact with the skin during use. If contacting with skin, rinse repeatedly with water or soapy water as soon as possible.

(5) The performance of the Kit is not affected though the ice pack equipped with the Kit has melted upon receipt. The Kit can be placed at 37℃ for 2 days.

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

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