High Activity Brain Tumor Enzymatic Digestion Kit Instructions

Product Information

Product Name	Model	Specification
High Activity Brain Tumor Enzymatic Digestion Kit	DHBTE-2508	25 T

Product Description

High Activity Brain Tumor Enzymatic Digestion Kit (the "Kit") can prepare human brain tumors (clinical brain tumor and implanted brain tumor tissue) and mouse brain tumors (spontaneous brain tumor and implanted brain tumor tissue) 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 and primary cell culture.

Main principle: The Kit can be used in combination with RWD Single Cell Suspension Dissociator. The dissociator mainly plays a role of mechanical dissociation, while the Kit digests the tissue by enzymatic digestion. After dissociation, the cell suspension is filtered with cell strainers to remove tissue residues to obtain single cell suspension.

Components

Product Name	Components	Quantity	Storage Condition
High Activity Brain Tumor Enzymatic Digestion Kit	Enzyme A Reagent (powder)	1 vial	2℃ ~ 8℃
	Enzyme B Reagent (powder)	1 vial	-25℃ ~ -15℃
	Enzyme C Reagent (powder)	1 vial	2℃ ~ 8℃
	Buffer A (solution)	1 vial	2℃ ~ 8℃
	Buffer B (solution)	1 vial	2℃ ~ 8℃

Test Capacity

Recommendation for single process of tissue:

Tissue Type	Capacity	Initial Sample Dosage
Brain Tumor Tissue	25 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, mix 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	RPMI 1640 or DMEM Medium	PBS Buffer	HBSS Buffer (with Ca^{2+} and Mg^{2+})
	Red Blood Cell Lysis Buffer (Optional)		
Consumable	Tissue Processing Tube (RWD)	Heater (RWD # HJ-400)	70 μm Cell Strainer
	0.22 μm Syringe Filter (Optional)		
Instrument	Single Cell Suspension Dissociator (RWD)	Vortex Oscillator	Constant Temperature Oscillator

Operation

Preparation

(1) Preparation of enzyme A solution: Dissolve the powder of the enzyme A reagent with 1.5 mL HBSS (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 0.75 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 1.5 mL HBSS (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℃.

(4) Preparation of enzyme mixture: Prepare the mixture according to the table below, and the enzyme mixture is freshly prepared just before use. The enzyme mixture can be used to process up to 500 mg human or mouse brain tumor tissue. When processing human or mouse brain tumor tissue greater than 500 mg, the amount of tissue processing tube should be increased. If subsequent cell culture is required, the enzyme mixture should be sterile-filtered with a 0.22 μm syringe filter.

Enzyme Mixture			
Buffer A 1875 μL	Enzyme A 50 μL	Enzyme B 25 μL	Enzyme C 50 μL

Mechanized Protocol

(1) After stripping the brain tumor tissue, place and store the brain tumor tissue in a petri dish containing RPMI 1640 medium or DMEM medium or PBS buffer with solution overhead the brain tissue. Remove the blood capillaries gently from the above tissue as much as possible by using a small curved ophthalmic forceps and cut the tissue into small pieces of 2 ~ 4 mm size using the ophthalmic scissors.

(2) Place the prepared enzyme mixture in a 37°C constant temperature oscillator, rotate it continuously at 50 ~ 100 rpm and incubate for 25 ~ 30 min.

(3) Weigh the tissue and transfer them to the tissue processing tube containing enzyme mixture.

(4) 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.

(5) If the single cell suspension dissociator is used, run the program **M_BTumor_Heater_1** for both the soft and hard brain tumor tissue; for softer brain tumor tissue, run the program **M_BTumor_Heater_1**; for harder brain tumor tissue, run the program **M_BTumor_Heater_2**.

(6) After the program ends, remove the tissue processing tube from the single cell suspension dissociator. Then, use the 1 mL pipette to blow the cell suspension 8 ~ 10 times.

(7) Wet the 70 μm cell strainer with 1 mL of RPMI 1640 medium or DMEM medium or PBS buffer, filter the cell suspension by the cell strainer, and collect the cell suspension to the 50 mL centrifuge tube.

(8) Rinse the tissue processing tube with 10 mL RPMI 1640 medium or DMEM medium or PBS buffer. Filter the suspension by the 70 μm cell strainer and collect it to the 50 mL centrifuge tube in step (7).

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

(Optional) Removal of red blood cells

Resuspend the cells collected in step (9) with 1 ~ 2 mL of red blood cell lysis buffer, then incubate on ice for 2 ~ 3 min, followed by resuspension with 10 mL of RPMI 1640 medium or DMEM medium or PBS buffer. Centrifuge the cell suspension at 300×g for 10 min and discard the supernatant completely.

(10) Resuspend the cells to required volume with RPMI 1640 medium or DMEM medium or PBS buffer or

other buffer for subsequent experiments.

Manual Protocol

(1) Process the tissue as mentioned in step (1) of ***"Mechanized Protocol"***.

(2) Transfer the tissue pieces to a 50 mL centrifuge tube containing enzyme mixture and tighten the cap. (Before transferring the tissue pieces, the prepared enzyme mixture should be incubated in the 37°C constant temperature oscillator at 50 ~ 100 rpm for 25 ~ 30 min.)

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

(3) Incubate the tissue in the 37°C constant temperature oscillator at 150 rpm for 15 min and blow them up and down 20 times by using a 1 mL pipette which the tip is cut off about 5 mm.

(4) Add 10 mL RPMI 1640 medium or DMEM medium to stop the digestion.

(5) Follow the steps in ***"Mechanized Protocol"*** from step (6) to (10). When filtering the tissue pieces, residual tissue can be blew and ground on the sieve by the 1 mL pipette with appropriate amount 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.

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

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