High Activity Tumor Tissue Enzymatic Digestion Kit (Human) Instructions

Product Information

Product Name	Model	Specification
High Activity Tumor Tissue Enzymatic Digestion Kit (Human)	DHTEH-2505	25 T

Product Description

High Activity Tumor Tissue Enzymatic Digestion Kit (Human) (the "Kit") can prepare primary tumor tissue or transplanted tumor tissue of human 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 of tumor cell or tumor infiltrating lymphocytes (TIL), 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 tumor 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 Tumor Tissue Enzymatic Digestion Kit (Human)	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
Human Tumor Tissue	25 T	0.05 ~ 1.0 g to be processed per time (include)

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+})	RPMI 1640 or DMEM Medium	PBS
	Red Blood Cell Lysis Buffer (optional)		
Consumable	Tissue Processing Tube (RWD)	Heater (RWD # HJ-400)	70 μm Cell Strainer
Consumable	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 6 mL HBSS buffer (with Ca^{2+} and Mg^{2+}), 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, thawing and shaking (Centrifuge tube can be used to help dissolve the powder).

(2) Preparation of enzyme B solution: Dissolve the powder of the enzyme B reagent with 9 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, thawing and shaking.

(3) Preparation of enzyme C solution: Dissolve the powder of the enzyme C reagent with 1 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, thawing and shaking.

(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 up to 1.0 g tumor tissue. When processing tumor tissue greater than 1.0 g, 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 or 5 mL in total.

Tissue Weight	Enzyme Mixture			
0.05 ~ 0.2 g	HBSS buffer (with Ca^{2+} and Mg^{2+}) 2.24 mL	Enzyme A 100 μL	Enzyme B 150 μL	Enzyme C 12.5 μL
0.2 ~ 1 g	HBSS buffer (with Ca^{2+} and Mg^{2+}) 4.48 mL	Enzyme A 200 μL	Enzyme B 300 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) Rinse the tumor tissue, then cut the tissue into pieces of 2 ~ 4 mm, put them in the petri dish containing PBS or RPMI 1640 or DMEM medium for temporary storage, and weigh target weight of the tissue pieces by the electronic balance scale.

⚠ Note: When cutting the tissue, observe the tissue texture and remove the fat, connective tissue and necrotic core area as much as possible.

(3) Transfer the tissue pieces 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.

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

(5) For soft tumor tissue, run the program **H_Tumor_Heater_1**; for medium hard tumor tissue, run the program **H_Tumor_Heater_2**; for hard tumor tissue, run the program **H_Tumor_Heater_3**. The type of tumor is shown in the table below.

Tumor Type	Example
Soft	Melanoma, Ovarian carcinoma, Colonic carcinoma, Nasopharyngeal carcinoma, Clear cell renal cell adenocarcinoma
Medium	Lung carcinoma, Prostatic carcinoma

Tumor Type	Example
Hard	Breast carcinoma, Pancreatic carcinoma, Hepatocellular carcinoma, Head and Neck Squamous Cell Carcinoma (HNSCC)

(6) After the program is finished, remove the tissue processing tube from the single cell suspension dissociator.
⚠ Note: When processing hard tumor tissues, there may be tissue residues left. To increase the cell yield, collect the remaining tissue pellets and transfer the digested supernatant to a new centrifuge tube. Then, aspirate 4 mL RPMI 1640 or DMEM and mix it with the remaining tissue pieces in the tissue processing tube. Run the program **Mouse_Tumor_3** on the single cell suspension dissociator. Finally, combine the obtained cell suspension with the digested supernatant in the previous centrifuge tube.

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

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

(9) Centrifugate the cell suspension at 500×g for 5 min and discard the supernatant completely.

(Optional) Removal of red blood cells

If it is necessary to remove the red blood cells, resuspend the cells collected in step (9) with 1 mL red blood cell lysis buffer. Then, incubate the suspension on ice for about 3 min, followed by resuspension with 6 mL PBS or RPMI 1640 or DMEM medium. Centrifugate the cell suspension at 500×g for 5 min and discard the supernatant completely.

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

Manual Protocol

(1) Prepare the enzyme mixture in the centrifuge tube following step (4) in ***"Preparation"***.

(2) Rinse the tumor tissue, then cut the tissue into pieces of 2 ~ 4 mm, put them in the petri dish containing PBS or RPMI 1640 or DMEM medium for temporary storage, and weigh target weight of the tissue pieces by the electronic balance scale.
⚠ Note: When cutting the tissue, observe the tissue texture and remove the fat, connective tissue and necrotic core area as much as possible.

(3) Transfer the tissue pieces to the centrifuge tube containing enzyme mixture.

(4) For soft tissue, place the centrifuge tube and incubate the tissue in the 37°C constant temperature oscillator; for hard tissue, place the centrifuge tube and incubate the tissue in the 37°C constant temperature oscillator at 50 rpm.

(5) The time of incubation should be about 40 min. During the incubation, oscillate the suspension for 20 s each 10 min (at intermediate speed). It should be oscillated 4 ~ 5 times in total. During the digestion, it is suggested to observe carefully the condition to avoid over-digestion.

(6) For the tissue residue, oscillate the tissue again in the vortex oscillator. Open the centrifuge tube, blow the cell suspension 20 times by the l mL pipette, which the tip is cut off 0.5 cm.

(7) Follow the steps in ***"Mechanized Protocol"*** from step (7) to (10). Tissue residue can be blown and ground by the l mL pipette aspirated with appropriate 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) Processing 0.05 ~ 0.2 g (include) tumor tissue requires approximately 2.5 mL enzyme mixture for enzymatic digestion; processing 0.2 ~ 1.0 g (include) tumor tissue requires approximately 5 mL enzyme mixture for enzymatic digestion

(4) To analyze the TIL, it is suggested to decrease the proportion of enzyme B in enzyme mixture to 20% (if the tissue weight＞0.2 g, only 60 μL enzyme B to be added). The increase of the volume of enzyme B can better help protect the cell surface epitopes, but will slightly influence the cell yield and cell viability.

(5) 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.

RWD Life Science Co., Ltd.
Add: 10410 Corporate Drive, Sugar Land, TX 77478, USA
Add: (Floor 9, 19&20 Building 7A, Floor 9 Building 7D) Room 1901, Building 7A, International Innovation Valley, Dashi 1st Road, Xili Community, Nanshan District, Shenzhen 518000, Guangdong, P. R. China
Web: www.rwdstco.com E-Mail: service@rwdls.com
Tel: 0086-755-86111281 001-858-900-6602 (USA)

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