High Activity Tumor Tissue Enzymatic Digestion Kit (Human) - Instructions

1 Product Information

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

2 Description

The High Activity Tumor Tissue Enzymatic Digestion Kit（Human） can be used to prepare single cell suspensions from primary tumor tissue or transplanted tumors gently, rapidly and efficiently. This optimization scheme can help obtain as many highly-viable single cell samples as possible while maintaining the important surface epitopes of cells. The prepared single cell suspension can continue to be used for downstream experimental applications such as culturing tumor cells or tumor-infiltrating lymphocytes (TILs) or sorting cells.

Main principle: Tumor tissue is prepared as a single cell suspension by a combination of mechanical shearing and enzymatic digestion of the extracellular matrix to keep the tissue structurally intact. The RWD Life Science Single Cell Suspension Dissociator is chiefly used for mechanical dissociation, while the High Activity Tumor Tissue Enzymatic Digestion Kit（Human） mainly digests the tissue through enzymatic hydrolysis. After dissociation, the sample is filtered using a cell strainer to remove tissue residues to obtain a single cell suspension, and the obtained cells can be used immediately for subsequent experiments, such as primary cell culture, cell sorting, single cell sequencing, etc.

3 Components

5 bottles of reagents in total, including:

1 bottle of Enzyme A powder;
1 bottle of Enzyme B powder;
1 bottle of Enzyme C powder;
1 bottle of Buffer B reagent (solution);
1 bottle of Buffer C reagent (solution).

4 Test Capacity

It can be used to dissociate tumor tissue 25 times, with 0.05 ~ 1.0 g of tumor tissue per treatment. The volume of enzyme mixture added varies depending on the weight of the treated tissue. Please refer to **Table 4-1** for details.

Table 4-1

Weight of Tumor Sample	Enzyme Mixture
0.05 ~ 0.2 g (not included)	2.2 mL RPMI 1640 / DMEM 100 μl Enzyme A + 150 μL Enzyme B + 12.5 μL Enzyme C
0.2 ~ 1 g (not included)	4.5 mL RPMI 1640 / DMEM 200 μL Enzyme A + 300 μL Enzyme B + 25 μL Enzyme C

If subsequent cell culture is required, sterile filtration with a 0.22 μm filter after the preparation of the enzyme mixture. After filtration, ensure that the enzyme mixture has a total volume of 2.5 mL or 5 mL. When the tumor tissue weighs more than 1 g, we recommend aliquoting it into multiple tissue processing tubes for the experiment.

5 Transport and storage

Transport in ice packs at 2 ~ 8°C;

Upon receiving the kit, store the kit contents in separate places according to the components;

Enzyme C reagent is stored at -25 ~ -15°C, and the rest components are stored at 2 ~ 8°C, with validity period of 12 months.

6 Reagent and Instrument Requirements

RPMI 1640 or DMEM;
Hanks (containing calcium and magnesium) (Solarbio: H1020);
(Optional) Red blood cell lysis buffer (Solarbio: R1010);
70 μm cell strainers;
Constant temperature oscillator;
DSC-400 / DSC-800 single cell suspension dissociator (RWD);
Tissue processing tube* (RWD);
HJ-400 heater (RWD);
0.22 μm syringe filters.

7 Method for use

7.1 Reagent Preparation

1) Preparing Enzyme A solution: Add 6 mL of Hanks (containing calcium and magnesium) to the vial containing Enzyme A powder and mix them well;
2) Preparing Enzyme B solution: Add 9 mL of Buffer B to the vial containing Enzyme B powder and mix them well;
3) Preparing Enzyme C solution: Add 1 mL of Buffer C to the vial containing Enzyme C powder and mix them well.

The 15 mL centrifuge tube can be used to assist in dissolving Enzyme A and Enzyme B solutions. The dissolved components can be aliquoted into EP tubes and stored at -25 ~ -15 °C to avoid repeated freezing and thawing. The dissolved components can keep stable for 6 months.

7.2 Protocol for Gentle Enzymolysis of Tumor Tissue

7.2.1 Application of DSC-400 / DSC-800 Single Cell Suspension Dissociator (HJ-400 Heater Included)

1) Prepare the enzyme mixture by adding the corresponding enzymatic hydrolysis reagents to the tissue processing tube as described in **Table 4-1** above;
2) After rinsing the tumor tissue, cut it into small pieces 2 ~ 4 mm, temporarily store them in a vessel containing RPMI 1640 or DMEM, and weigh the tissue pieces of the target mass on an electronic balance;

   ⚠ **Note: Sample texture can be observed during shearing. If possible, fat, connective tissue and core necrotic areas of the tumor tissue samples should be cut during processing.**
3) Transfer the weighed tumor tissue to a tissue processing tube containing the enzyme mixture;
4) Tighten the tissue processing tube. Invert and install the tube into the sleeve of the DSC-400 / DSC-800 single cell suspension dissociator (a heater included).

   ⚠ **Note: Make sure the sample material is in the area where the rotor/stator is located.**
5) Run the program "**H_Tumor_Heater_1**" for soft tumor tissue or "**H_Tumor_Heater_2**" for medium-hard tumor tissue or "**H_Tumor_Heater_3**" for hard tumor tissue. For the type of tumors, please refer to **Table 7-1**;

Table 7-1

Tumor Types	Examples
Soft tissue	Melanoma, ovarian cancer, colon cancer, nasopharyngeal carcinoma, renal clear cell adenocarcinoma, etc
Medium-hard tissue	Lung cancer, prostate cancer, etc
Hard tissue	Breast cancer, pancreatic cancer, liver cancer, head and neck squamous cell carcinoma (HNSCC), etc

6) After the program ends, proceed with step 12) in 7.2.2 until the operation finishes.

7.2.2 Application of DSC-400 / DSC-800 Single Cell Suspension Dissociator Only

1) Prepare the enzyme mixture by adding the corresponding enzymatic hydrolysis reagents to the tissue processing tube as described in **Table 4-1** above;
2) After rinsing the tumor tissue, cut it into small pieces 2 ~ 4 mm, temporarily store them in a vessel containing RPMI 1640 or DMEM, and weigh the tissue pieces of the target mass on an electronic balance;

   ⚠ **Note: Sample texture can be observed during shearing. If possible, fat, connective tissue and core necrotic areas of the human tumor tissue samples should be cut during processing.**
3) Transfer the weighed tumor tissue to a tissue processing tube containing the enzyme mixture;
4) Tighten the tissue processing tube. Invert and install the tube into the sleeve of the DSC-400 / DSC-800 single cell suspension dissociator.

   ⚠ **Note: Make sure the sample material is in the area where the rotor/stator is located.**
5) Run the program "**Human_Tumor_1**" first for all three types of tissue. Remove the tissue processing tube after doing so;
6) Place the tissue processing tube into a constant temperature oscillator, and then perform continuous rotation and incubation at 37°C at 100 rpm for 30 minutes;

   ⚠ **Note: Always keep the tissue processing tube upside down so that no residual tissue will adhere to the tube wall;**
7) After incubation, invert and install the tissue processing tube into the sleeve of the DSC-400 / DSC-800 single cell suspension dissociator.

   ⚠ **Note: Make sure the sample material is in the area where the rotor/stator is located.**
8) Run the program suitable for the type of tumor tissue being processed according to **Table 7-2**:

Table 7-2

Tumor Types	Program Running
Soft tissue	Human_Tumor_2
Medium-hard tissue	Human_Tumor_2
Hard tissue	Human_Tumor_1

9) At the end of the program, remove the tissue processing tube. Place the tube into a constant temperature oscillator, and then perform continuous rotation and incubation at 37℃ at 100 RPM for 30 minutes;

   ⚠ **Note: Always keep the tissue processing tube upside down so that no residual tissue will adhere to the tube wall.**
10) After incubation, invert and install the tissue processing tube into the sleeve of the DSC-400 / DSC-800 single cell suspension dissociator.

    ⚠ **Note: Make sure the sample material is in the area where the rotor/stator is located.**
11) Run the program suitable for the type of tumor tissue **being processed** according to **Table 7-3**:

Table 7-3

Tumor Types	Program Running
Soft tissue	Human_Tumor_3
Medium-hard tissue	Human_Tumor_2
Hard tissue	Human_Tumor_1

12) At the end of the program, remove the tissue processing tube from the DSC-400 / DSC-800 single cell suspension dissociator;

⚠ **Note: When hard tumors are dealt with, tissue residues may emerge. The remaining tissue pellet may be collected and the digested supernatant transferred to a new centrifuge tube to further increase the cell yield. Pipette 4 mL of RPMI 1640 or DMEM and mix it with the remaining tissue pieces in the tissue processing tube. Insert the tube into the sleeve of the DSC-400 / DSC-800 and run the program "Mouse_Tumor_3". Combine the obtained cell suspension with the digested supernatant from the previous centrifuge tube.**

13) Wet a 70 μm cell strainer with 1 mL RPMI 1640 or DMEM, filter the cell suspension sample with the wetted cell strainer, and collect the cell suspension into a 50 mL centrifuge tube;
14) Rinse the tissue processing tube with 10 mL RPMI 1640 or DMEM, filter through a 70 μm cell strainer, and collect into the 50 mL centrifuge tube from step 13);
15) Centrifuge the cell suspension at 500×g for 5 minutes and discard the supernatant completely;
16) Resuspend the cells to the desired volume with RPMI 1640 or DMEM for further experimental applications;
17) (Optional) If red blood cells are to be removed, resuspend the treated cells from step 16) using the red blood cell lysis solution (Solarbio: R1010), and then incubate them on ice for 3 minutes, followed by termination with 6 mL of RPMI 1640 or DMEM. Centrifuge the cell suspension at 500×g for 5 minutes and discard the supernatant completely.

8 Precautions

1) The shelf life of this kit is 12 months, and RWD will not guarantee the validity of expired products;
2) When culturing downstream cells after tissue dissociation, make sure that all operations are performed under sterile conditions;
3) 0.05 ~ 0.2 g tumor tissue requires 2.5 mL enzyme mixture for digestion, and 0.2 ~ 1.0 g tumor tissue requires 5 mL enzyme mixture for digestion;
4) For analysis of TILs, it is recommended to reduce the content of Enzyme B in the enzyme mixture to 20% (for example, if the tissue weighs > 0.2 g, add only 60 μL of Enzyme B). Doing so helps to protect cell surface epitopes better but may slightly reduce cell yield and viability.
5) Due to weather conditions, the performance of the kit will not be affected even if the ice packs are dissolved when the kit is received. The kit has been tested for transportation.

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

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