High Activity FFPE Tissue Enzymatic Digestion Kit-25T Instructions

Product Information

Product Name	Model	Specification
High Activity FFPE Tissue Enzymatic Digestion Kit-25T	DHFTE-2517	25 T

Product Description

High Activity FFPE Tissue Enzymatic Digestion Kit-25T (the "Kit") is designed to gently, rapidly, and efficiently prepare FFPE (formalin-fixed paraffin-embedded) tissue sections into single-cell suspensions. The optimized protocol maximizes the yield of viable single-cell samples while preserving critical antigenic epitopes, ensuring the integrity of cellular structures. The resulting single-cell suspensions are suitable for flow cytometry analysis, as well as other genetic or proteomic studies.

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

Product Components

Product Name	Components	Quantity	Storage
High Activity FFPE Tissue Enzymatic Digestion Kit-25T	Enzyme A	1 vial	-25 ~ -15℃
	Enzyme B	1 vial	-25 ~ -15℃
	Buffer C	1 vial	2 ~ 8℃
	Buffer D	1 vial	2 ~ 8℃
	Buffer E	1 vial	2 ~ 8℃

Test Capacity

Recommendation for single process of tissue:

Tissue Type	Capacity	Maximum Sample Dosage
FFPE Tissue Section (50 μm)	25 T	2 pieces/T

Storage & Transportation

- ✧ The Kit should be transported at 2 ~ 8℃.
- ✧ The Kit is separated into two packages due to different storage temperatures, 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 vigorous shaking.
- ✧ The Kit is valid for 12 months from the date of manufacture.

Reagents & Instruments

The reagents and instruments needed are as follows:

Reagents	Double Distilled Water, HBSS (with Ca^{2+} and Mg^{2+}), PBS, Ethanol (50%, 70%, 100%), Xylene
Consumables	70 μm Cell Strainer
Instruments	Centrifuge (RWD), Single Cell Suspension Dissociator (RWD), Heater (RWD), Tissue Processing Tube (RWD), Shaking Water Bath

Operation

Preparation

(1) Preparation of Enzyme A solution: Prepare the enzyme A solution by reconstitution of the lyophilized powder in the vial of enzyme A with 2.7 mL of HBSS (containing Ca^{2+} and Mg^{2+}). Prepare aliquots of appropriate volume to avoid repeated freeze-thaw-cycles. Store aliquots at -25 ~ -15 °C. This solution is stable for 6 months after reconstitution.

(2) Preparation of Enzyme B solution: Prepare the enzyme B solution by reconstitution of the lyophilized powder in the vial of enzyme B with 2.7 mL of Buffer C. Prepare aliquots of appropriate volume to avoid repeated freeze-thaw-cycles. Store aliquots at -25 ~ -15 °C. This solution is stable for 6 months after reconstitution.

(3) Preparation of Enzyme Mix: Prepare enzyme mix according to the table below. The enzyme mix should be freshly prepared and used immediately. Volumes given below are for up to two FFPE tissue sections (50 μm each) in 2.5 mL enzyme mix per tube. If more tissues are to be processed, more tissue processing tubes are needed.

Enzyme Mix		
Buffer E 2.3 mL	Enzyme A 100 μL	Enzyme B 100 μL

Sample Pretreatment

⚠ Note: Pre-heat shaking water bath and 3 mL of Buffer D to 80 °C for step (10).

(1) Add 1 ~ 2 pieces of FFPE tissue sections (50 μm) into the 5 mL centrifuge tube.

(2) Add 3 mL of xylene and incubate at room temperature for 10 minutes, then remove the liquid.

⚠ Note: Xylene can be substituted with other tissue deparaffinization solutions.

(3) Repeat step (2) twice.

(4) Add 3 mL of 100% ethanol and incubate at room temperature for 30 seconds, then remove the liquid.

(5) Add 1 mL of 100% ethanol and incubate at room temperature for 30 seconds, then remove the liquid.

(6) Add 1 mL of 70% ethanol and incubate at room temperature for 30 seconds, then remove the liquid.

(7) Add 1 mL of 50% ethanol and incubate at room temperature for 30 seconds, then remove the liquid.

(8) Add 1 mL of double distilled water and incubate at room temperature for 30 seconds, then remove the liquid.

(9) Add 1 mL of unheated Buffer D to the tissue processing tube, and incubate at room temperature for 30 seconds, then remove the liquid.

(10) Add 3 mL of preheated Buffer D to the tube, place the tube in the pre-heated water bath and incubate at 80°C for 75 minutes.

(11) Remove the tube from the water bath and allow it to cool at room temperature for 20 minutes, then remove

the liquid.

(12) Add 1 mL of Buffer E and incubate at room temperature for 30 seconds, then remove the liquid.

Mechanized Protocol

(1) Transfer the sample to a tissue processing tube after completing the steps of ***Sample Pretreatment***.

(2) Add 2.5 mL of enzyme mix to the tube, tighten the cap, invert and mount it in the bushing of the single cell suspension dissociator with the heater.

⚠ Note: During installation, ensure that the sample is always submerged in the enzyme mix.

(3) Run the **FFPE_Heater_1** program.

(4) After the program is completed, remove the tissue processing tube from the single cell suspension dissociator and centrifuge it briefly at 300×g, 4℃.

(5) Resuspend the cell suspension, filter the cell suspension through the 70 μm cell strainer, and rinse the cell strainer with 1 mL of pre-cooled PBS, then collect it to a new tube.

(6) Centrifuge the filtered cell suspension at 1000×g for 5 minutes at 4℃, then discard the supernatant.

(7) Resuspend the cell pellet with 1 mL of pre-cooled PBS buffer and centrifuge at 1000×g for 5 minutes at 4℃, then discard the supernatant.

(8) Resuspend the cells with 0.5 mL of pre-cooled PBS buffer.

Manual Protocol

(1) Add 2.5 mL of enzyme mix to the tube in step (12) of ***Sample Pretreatment***, and seal the tube.

(2) Place the tube in a shaking water wath and incubate at 37℃ with shaking at 100 rpm for 50 minutes.

⚠ Note: It is recommended to place the tube horizontally to ensure full contact between the sample and enzyme mix.

(3) Remove the tube and centrifuge it briefly at 300×g, 4℃.

(4) Resuspend the cell suspension, filter the cell suspension through the 70 μm cell strainer, and rinse the cell strainer with 1 mL of pre-cooled PBS, then collect it to a new tube.

(5) Centrifuge the filtered cell suspension at 1000×g for 5 minutes at 4℃, then discard the supernatant.

(6) Resuspend the cell pellet with 1 mL of pre-cooled PBS buffer and centrifuge at 1000×g for 5 minutes at 4℃, then discard the supernatant.

(7) Resuspend the cell pellet with 0.5 mL of pre-cooled PBS buffer.

Precautions

(1) The Kit is valid for 12 months and RWD shall not guarantee the validity of expired products.

(2) The Kit has passed the transportation test. The performance of the Kit is not affected though the ice pack equipped with the Kit has melted upon receipt.

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