High Activity Brain Tumor Enzymatic Digestion Kit

1 Product Information

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

2 Description

This kit is designed to provide enzymatic solution for brain tumor tissue sample processing. It can gently, quickly and efficiently dissociate human brain tumors (including clinical and implanted brain tumor tissues) and mouse brain tumors (mouse spontaneous and implanted brain tumor tissues) into single-cell suspensions. This optimization scheme can help obtain as many single cell samples with high cell viability as possible, while maintaining the important surface epitopes of cells. The obtained single cell suspension can continue to be applied in downstream experiments such as cell sorting and primary cell culture, etc.

Main principle: human and mouse brain tumors are dissociated into single-cell suspensions by a combination of mechanical dissociation and enzymatic digestion of the extracellular matrix (to maintain structural integrity of the tissue). RWD single cell suspension dissociator mainly plays the role of mechanical dissociation, while the High Activity Brain Tumor Enzymatic Digestion Kit mainly digests the tissue by enzymatic digestion. After dissociation, the sample is filtered with a cell filter to remove tissue residues in the sample to obtain a single-cell suspension, and the cells obtained can be used immediately for subsequent experiments, such as primary cell culture, cell sorting and single-cell sequencing etc.

3 Components

5 vials of reagents, including

1 vial of Enzyme A reagent (lyophilized powder)
1 vial of Enzyme B reagent (lyophilized powder)
1 vial of Enzyme C reagent (lyophilized powder)
1 vial of Buffer A reagent (solution)
1 vial of Buffer B reagent (solution)

4 Capacity

For 25 digestions of human or mouse brain tumor tissue, with 50 ~ 500 mg of human or mouse brain tumor tissue digested each time.

5 Transportation and storage

Transport in ice packs at 2 to 8°C;

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

6 Requirements for reagents and instruments

HBSS (containing Ca^{2+} and Mg^{2+})
RPMI 1640 or DMEM
70 μm cell filter
Constant temperature oscillator
DSC-400/ DSC-800 Single cell suspension dissociator (RWD)
Tissue processing tube* (RWD)
HJ-400 Heater (Optional, RWD)

7 Method for use

7.1 Reagent preparation

7.1.1 Dissolve enzyme dry powder

1) Prepare Enzyme A solution: dissolve the powder in the Enzyme A reagent bottle with 1.5 mL HBSS (containing Ca^{2+} and Mg^{2+}) at 37 °C water bath and mix well. After dissolution, sub-pack the solution directly, followed by frozen storage at -25 ~ -15°C to avoid repeated freezing and thawing and vibrate agitation. The enzyme solution can be stored stably for 6 months at -25 ~ -15°C.

2) Prepare Enzyme B solution: dissolve the powder in the Enzyme B reagent bottle with 0.75 mL Buffer B. After dissolution, sub-pack the solution directly, followed by frozen storage at -25 ~ -15°C to avoid repeated freezing and thawing and vibrate agitation. The enzyme solution can be stored stably for 6 months at -25 ~ -15℃.
3) Prepare Enzyme C solution: dissolve the powder in the Enzyme C reagent bottle with 1.5 mL HBSS (containing Ca^{2+} and Mg^{2+}). After dissolution, sub-pack the solution directly, followed by frozen storage at -25 ~ -15°C to avoid repeated freezing and thawing and vibrate agitation. The enzyme solution can be stored stably for 6 months at -25 ~ -15℃.

7.1.2 Prepare enzyme mixture

Prepare mix 1 according to the table below, and the enzyme mixture is freshly prepared just before use. The Enzyme mix 1 prepared below can be used for 50 ~ 500 mg human or mouse brain tumor tissue. When working with more than 500 mg of human or mouse brain tumor tissue, determine the weight and scale up all reagent volumes and total Enzyme mix 1 volumes accordingly. If subsequent cell culture is required, the enzyme mixture should be sterile-filtered (e.g., filtration with a 0.22 μm syringe filter). After filtration, the total volume of enzyme mixture should be 2mL.

Enzyme mix 1			
Enzyme A 50 μL	Buffer A 1875 μL	Enzyme B 25 μL	Enzyme C 50 μL

7.1.3 Activation of enzyme reagents

The prepared Enzyme mix1 was placed in a 37 °C constant temperature oscillator, rotate it continuously at 50 ~ 100 rpm and incubate for 25 ~ 30 min.

7.2 Tumor tissue gentle enzymatic digestion

7.2.1 DSC-400 / DSC-800 Single cell suspension dissociator with HJ-400 heater is used

1) After stripping the brain tumor tissue, place and temporarily store the brain tumor tissue in a petri dish containing HBSS (containing Ca^{2+} and Mg2+) or RPMI 1640 or DMEM with solution overhead the brain tissue, and remove blood capillaries gently from the above tissue as much as possible by using small curved ophthalmic forceps. The brain tumor tissue samples were then cut into small pieces of 2 ~ 4 mm size using ophthalmic scissors.
2) Weigh the brain tumor tissue and add Enzyme mix 1 incubated in step 7.1.3 to a tissue processing tube. Then transfer the brain tumor tissue to the tissue processing tube.
3) Tighten the tissue processing tube, invert it and mount it into the channel of the DSC-400/DSC-800 single cell suspension dissociator, and install the heater (Note: make sure the sample is located in the rotor/stator area).
4) Run program **M_BTumor_Heater_1**.
5) After the program ends, proceed with step 11) in 7.2.2 until the operation finishes.

7.2.2 DSC-400 / DSC-800 Single cell suspension dissociator without HJ-400 heater is used

1) After stripping the brain tumor tissue, place and temporarily store the brain tumor tissue in a petri dish containing HBSS (containing Ca^{2+} and Mg^{2+}) or RPMI 1640 or DMEM with solution overhead the brain tissue, and remove blood capillaries gently from the above tissue as much as possible by using small curved ophthalmic forceps. The brain tumor tissue samples were then cut into small pieces of 2 ~ 4 mm size using ophthalmic scissors.
2) Weigh the brain tumor tissue. Add Enzyme mix 1 incubated in step 7.1.3 to a tissue processing tube. Then transfer the brain tumor tissue to the tissue processing tube.
3) Tighten the tissue processing tube, invert it, and mount it into the channel of the DSC-400/DSC-800 single cell suspension dissociator, and install the heater.
   ⚠Note: make sure the sample is located in the rotor/stator area.
4) Run program **Human_Tumor_2**.

5) After the program running is completed, remove the tissue processing tube from the DSC-400/DSC-800 single cell suspension dissociator. Put the tissue processing tube in the constant temperature oscillator, rotate continuously at 100 rpm and incubate for 15 minutes at 37°C.
   ⚠Note: make sure the tissue processing tube is inverted to avoid the waste of tissues.
6) After incubation, invert the tissue processing tube and mount it into the channel of the DSC-400/DSC-800 single cell suspension dissociator.
   ⚠Note: make sure the sample is located in the rotor/stator area.
7) Run the program **Human_Tumor_3**.
8) After the program running is completed, remove the tissue processing tube from the DSC-400/DSC-800 single cell suspension dissociator. Put the tissue processing tube in the constant temperature oscillator, rotate continuously at 100 rpm and incubate for 10 minutes at 37°C.
   ⚠Note: make sure the tissue processing tube is inverted to avoid the waste of tissues.
9) After incubation, invert the tissue processing tube and mount it into the channel of the DSC-400/DSC-800 single cell suspension dissociator.
   ⚠Note: make sure the sample is located in the rotor/stator area.
10) Run the program **Mouse_Brain_1**.
11) At the end of the program, remove the tissue processing tube from the DSC-400/DSC-800 single cell suspension dissociator. Blow the mixed cell suspension 8 ~ 10 times with a 1 mL pipette.
   ⚠Note: When hard brain 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 **Human_Tumor_2**. Combine the obtained cell suspension with the digested supernatant from the previous centrifuge tube.
12) Wet the 70 μm cell filter with 1 mL RPMI 1640 or DMEM medium, filter the cell sample with the wetted 70 μm cell filter and collect the cell suspension in a 50 mL centrifuge tube.
13) Rinse the tissue processing tube with 10 mL of RPMI 1640 or DMEM and filter with a 70 μm filter, and collect in a 50 mL centrifuge tube as described in Step 12).
14) Centrifuge the cell suspension at 300×g for 10 min and discard the supernatant completely.
15) (Optional) To remove red blood cells
   Resuspend the cells resuspended in step 14) with 2 mL of 1×Red blood cell lysis buffe (Biolegend：#420301), then incubate on ice for 2 ~ 3 min, followed by resuspension with 12 mL of RPMI 1640 or DMEM, centrifuge the cell suspension at 300×g for 10 min, and discard the supernatant completely.
16) Resuspend the cells with RPMI 1640 or DMEM or other buffer to the required volume for subsequent experiments.

8 Precautions

1) This kit is valid for 12 months, and RWD shall not guarantee the validity of expired products.
2) When downstream cell culture is carried out after tissue dissociation, make sure that all operations are performed under sterile conditions.
3) The Enzyme A reagent stock solution needs to be incubated in a water bath at 37 °C for 3 ~ 5 minutes, then completely dissolved and prepared into enzyme mix 1.
4) Each tissue processing tube can process a maximum of 500 mg of brain tumor tissue.
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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