Liver Tissue Single-Cell Dissociation Enzyme

L1507367

Storage: 2~8℃.

Introduction:

This product is specifically designed for preparing single-cell suspensions from liver tissues of humans, mice, rats, and other animals. Through the gentle enzymatic hydrolysis of a multi-component composite enzyme, liver tissues are efficiently dissociated into single-cell suspensions, achieving high yield while maintaining high cell viability. The resulting single cells are suitable for downstream experiments including single-cell sequencing, flow cytometry analysis, flow cytometric sorting, primary cell culture, and organoid 3D culture.

Component List

L1507367	Component	50T	Storage	Digestion Capacity per 1T (mL)	Digestion Time
L1507367A	Single-Cell Dissociation Enzyme Powder A	50T	2~8℃ before reconstitution; -20℃ after reconstitution	50-150mg	15-30min
L1507367B	Dissociation Enzyme Reconstitution Buffer B	50mL	2~8℃		

Product Advantages:

1. Ready-to-Use (One-Step Operation):

Simply reconstitute the provided enzyme powder with the supplied buffer for immediate use in downstream experiments. Unlike imported competitors that require separate reconstitution of 3-4 enzymes in different solutions, volume calculation, and mixing, this product eliminates the need for weighing, complex calculations, and multi-step operations—significantly reducing the risk of errors.

2. Multi-Component Composite Enzyme:

Independently developed and manufactured, this product contains over 8 types of composite enzymes tailored to the extracellular matrix components of different tissues. It achieves more efficient digestion of intercellular matrices compared to single-use collagenase IV, DNase, or 3-4 component mixed enzymes from imported brands. Key benefits include shorter digestion time, higher cell yield, superior cell viability, intact antigen epitopes, and more competitive pricing due to independent R&D and production.

3. Short Digestion Time:

Leveraging over 8 types of self-developed tissue-specific dissociation enzymes, most organ tissues are fully dissociated within 10-15 minutes. In contrast, single collagenase IV, DNase, or imported mixed enzymes typically require ~1 hour, and sometimes 2-3 hours, for complete

dissociation.

4. High Cell Yield:

The multi-component composite enzyme ensures thorough digestion of intercellular matrices, maximizing the release of single cells with minimal tissue clump residues—resulting in higher overall cell yield.

5. Superior Cell Viability:

Tissue-specific composite enzymes enable gentle dissociation, causing less cell damage than trypsin. Combined with shorter digestion time, the product minimizes in vitro cell exposure and avoids over-digestion, preserving high cell viability and intact antigen epitopes.

6. Intact Antigen Epitopes:

Efficient digestion reduces both processing time and in vitro cell exposure, minimizing tissue/cell damage while maintaining structural integrity and high viability. Intact antigen epitopes facilitate antibody binding and staining, making the dissociated single cells ideal for flow cytometry analysis and sorting.

Usage Instructions:

1. Reconstitute Enzyme Powder:

Perform this step when first using the kit:

Centrifuge the vial of Enzyme Powder A briefly to collect all powder at the bottom.

Use a pipette to transfer Reconstitution Buffer B into the vial of Enzyme Powder A, repeatedly pipetting to resuspend the powder. For easier transfer, trim the tip of the pipette if necessary to ensure complete transfer of the powder into Buffer B.

Cap the vial tightly and invert to mix until the powder is fully dissolved (use a shaker if needed). The fully reconstituted enzyme solution will appear clear and slightly brown.

2. Filter and Aliquot Dissociation Enzyme:

After complete reconstitution:

For sterile tissue dissociation (e.g., cell culture), filter the enzyme solution through a 0.22μm sterile filter using a syringe, then aliquot.

Recommended aliquot volume: 5mL per vial (reuse vial A if desired).

Store aliquots at -20℃ or -80℃ immediately; stable for 6 months. Unused aliquots can be refrozen for future use, but avoid more than 3 freeze-thaw cycles (each cycle reduces enzyme activity—adjust volume accordingly to compensate for reduced activity).

3. Enzymatic Digestion Procedure:

(1) Pre-Experiment Preparation:

Prepare crushed ice; thaw the single-cell dissociation enzyme and digestion stop solution on ice.

Preheat the single-cell dissociation instrument (if used); pre-cool PBS, sterile surgical tools (ophthalmic scissors, forceps), 40μm sterile cell strainers, centrifuge tubes, and centrifuge.

(2) Tissue Collection:

Collect tissues under sterile conditions and immediately place in cold PBS. Process within 4 hours if possible; for longer storage, use High-Activity Tissue Preservation Solution.

(3) Tissue Washing:

Wash tissues 1-2 times with cold PBS to remove blood, necrotic tissue, and other impurities. Weigh and record the tissue mass.

(4) Tissue Mincing:

Transfer washed tissues to a dissociation tube and mince into 1-2mm³ pieces (mush-like consistency) using sharp ophthalmic scissors. Finer mincing ensures more efficient digestion and shorter processing time. If using a single-cell dissociation instrument, mincing can be less rigorous while maintaining good dissociation results.

(5) Tissue Digestion:

Add an appropriate volume of the reconstituted dissociation enzyme to the tube and invert to mix thoroughly with the minced tissue.

① If a tissue single-cell dissociation instrument is available, perform single-cell dissociation according to the instrument's operating specifications. This product is compatible with all commercial tissue single-cell dissociation instruments (both imported and domestic brands). However, note that its dissociation efficiency is higher than that of user-supplied enzymes or enzymes from other competitors, enabling at least a 50% reduction in dissociation time. Check the dissociation progress every 5-10 minutes—complete dissociation time varies by tissue (ranging from 5 to 50 minutes). Minimize enzymatic digestion time as much as possible while ensuring dissociation efficiency, and terminate digestion immediately once complete.

② If no tissue single-cell dissociation instrument is available, manual dissociation is also feasible:

Ensure tissues are minced strictly as required in the tissue mincing step.

Select a 1.5mL or 5mL EP tube based on the tissue quantity and enzyme volume added. Thoroughly mix the dissociation enzyme with the minced tissue.

Slightly trim the tip of a 1mL pipette with scissors, then vigorously pipette the tissue-enzyme mixture. If pipetting is blocked, the tissue is insufficiently minced—re-mince the tissue or trim the pipette tip further to ensure smooth repeated pipetting.

After 10+ rounds of vigorous pipetting, immediately incubate the tube at 37℃ in an incubator, water bath, or dry bath (a shaking incubator is preferred).

Vigorously pipette the mixture every 5 minutes until all tissue clumps are fully dissociated into a single-cell suspension.

(6) Single-Cell Filtration:

Once dissociation is complete, centrifuge the tube briefly (3 seconds) and place on ice.

Pipette the supernatant through a 40μm cell strainer into a new tube (perform all steps on ice).

(7) Stop Digestion:

Add an equal volume of Digestion Stop Solution or complete medium to terminate digestion.

(8) Cell Collection:

Centrifuge for 5 minutes and discard the supernatant.

(9) Red Blood Cell (RBC) Lysis:

Take an appropriate volume of red blood cell lysis buffer to resuspend the cell pellet.

Incubate on ice for 10 minutes, gently pipetting intermittently.
Centrifuge at 300g for 5 minutes at 4℃ and discard the supernatant.
(10) Cell Washing:
Centrifuge at 4℃ and discard the supernatant.
(11) Debris Removal:
If there is a high amount of debris, a high-efficiency debris remover can be used to eliminate it.
(12) Cell Viability Assessment:
Stain with trypan blue or AOPI; viable cell rate should exceed 85%.
(13) Cell Cryopreservation:
If downstream experiments are delayed, resuspend single cells in High-Efficiency High-Viability Cell Cryopreservation Solution and store directly at -80℃ (stable for years).

Notes:

For research use only.
For sterile culture applications, perform all operations in a biosafety cabinet to avoid contamination.
Digestion must be conducted at 37℃; all other steps should be performed on ice to strictly control temperature.
Adjust digestion time based on tissue quantity. Monitor dissociation progress closely and stop digestion as soon as complete to prevent cell damage.
For high-demand applications (e.g., single-cell sequencing), accelerate the entire process and proceed immediately to library preparation after dissociation.
Store reconstituted enzyme at -20℃ or -80℃ (stable for 6 months). Unused aliquots can be refrozen, but avoid more than 3 freeze-thaw cycles (each cycle reduces activity—increase enzyme volume if needed to maintain digestion efficiency).