

Cartilage Tissue Single-Cell Dissociation Enzyme

C1516123

Storage 2-8°C.

Shipping Low temperature transportation.

Introduction:

This product is specially designed for the preparation of single-cell suspensions from cartilage tissue such as ear cartilage of humans, mice, rats and other animals. Through mild enzymatic hydrolysis using a multi-component compound enzyme, it efficiently dissociates cartilage tissue into single-cell suspensions, delivering both high yield and high viability of single cells. The product is suitable for downstream applications including single-cell sequencing, flow cytometry analysis, flow cytometry sorting, primary cell culture, and 3D organoid culture.

Component List

C1516123	Component	50T	Storage	Digestion Capacity per 1T (mL)	Digestion Time
C1516123A	Single-Cell Dissociation Enzyme Powder A	50T	2~8°C before reconstitution; -20°C after reconstitution	50-150mg	15-30min
C1516123B	Dissociation Enzyme Reconstitution Buffer B	50mL	2~8°C		

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 0.22 μ m sterile filter using a syringe, then aliquot.

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

Store aliquots at -20°C or -80°C 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), 40 μ m 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°C 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 40 μ m 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°C and discard the supernatant.

(10) Cell Washing

Centrifuge at 4°C 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°C (stable for years).

Notes

1. For research use only.
2. For sterile culture applications, perform all operations in a biosafety cabinet to avoid contamination.
3. Digestion must be conducted at 37°C; 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.
4. For high-demand applications (e.g., single-cell sequencing), accelerate the entire process and proceed immediately to library preparation after dissociation.
5. Store reconstituted enzyme at -20°C or -80°C (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).