Trypsin Neutralizing Solution

PCS-999-004™

Description

Trypsin Neutralizing Solution is specifically formulated (5% FBS in phosphate-buffered saline without calcium and magnesium) to rapidly inactivate the concentration of trypsin found in the Trypsin-EDTA for Primary Cells solution.
Volume: 100 mL

Storage Conditions

Product format: Frozen
Storage conditions: -20°C or colder

Intended Use

This product is intended for laboratory research use only. It is not intended for any animal or human therapeutic use, any human or animal consumption, or any diagnostic use.

BSL 1

ATCC determines the biosafety level of a material based on our risk assessment as guided by the current edition of *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, U.S. Department of Health and Human Services. It is your responsibility to understand the hazards associated with the material per your organization's policies and procedures as well as any other applicable regulations as enforced by your local or national agencies.

Certificate of Analysis

For batch-specific test results, refer to the applicable certificate of analysis that can be found at www.atcc.org.

Handling Procedures

Each type of cell or cell line responds to Trypsin-EDTA for Primary Cells in a unique manner. For optimum results, continuously observe the cells during the dissociation process to prevent damage. For cell-specific information, please refer to the product sheet supplied with the cells or cell line.

1. Bring the DPBS, the Trypsin-EDTA for Primary Cells, and the Trypsin Neutralizing Solution to room temperature before use. Warm the complete growth medium to 37°C prior to use with the cells.
2. For each flask, carefully aspirate the spent media without disturbing the monolayer. If the cell culture medium contains serum, each flask should be rinsed with DPBS twice prior to adding the Trypsin-EDTA for Primary Cells.
3. Using 1 to 2 mL for every 25 cm^2, add the appropriate volume of trypsin-EDTA solution to each flask (e.g., each T-25 flask would be dissociated with 1 to 2 mL trypsin-EDTA).
4. Gently rock each flask to ensure complete coverage of the trypsin-EDTA solution over the cells, and then aspirate the excess fluid off of the monolayer; do not aspirate to dryness.
5. Observe the cells under the microscope. When the cells pull away from each other and round up (typically within about 3 to 6 minutes), remove the flask from the microscope and gently tap the culture flask from several sides to promote detachment of the cells from the flask. Do not over-trypsinize as this will damage the cells.
    a. Some strongly adherent cell types, such as keratinocytes, may take much longer and may require trypsinization at 37°C.
    b. Some cell types may require more vigorous tapping.
6. When the majority of cells appear to have detached, quickly add an equal

volume of the Trypsin Neutralizing Solution to each flask. Gently pipette or swirl the culture to ensure all of the trypsin-EDTA solution has been neutralized.

7. Transfer the dissociated cells to a sterile centrifuge tube and set aside while processing any remaining cells in the culture flask.
8. Add 3 to 5 mL DPBS to the tissue culture flask to collect any additional cells that might have been left behind.
9. Transfer the cell / DPBS suspension to the centrifuge tube containing the trypsin-EDTA-dissociated cells.
10. Repeat steps 8 and 9 as needed until all cells have been collected from all flasks.
11. Centrifuge the cells at 150 x g for 3 to 5 minutes.
    a. Do not over centrifuge cells as this may cause cell damage.
    b. After centrifugation, the cells should form a clean loose pellet.
12. Aspirate the neutralized dissociation solution and resuspend the cell pellet in 2 to 8 mL fresh, pre-warmed, complete growth medium.
13. Count the cells and seed new culture flasks at the recommended density.
14. Place newly seeded flasks in a 37°C, 5% CO_2 incubator and incubate for at least 24 to 48 hours before processing the cells further.

Quality Control Specifications

Bacterial and fungal testing: Not detected
Mycoplasma contamination: Not detected
Osmolality: 290 ± 20 mOsm/kg
pH: 7.5 ± 0.3
Functional tests: Each lot is assessed for cell passaging activity.
*A Certificate of Analysis (COA) is available upon request for each lot of Trypsin-EDTA for Primary Cells.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: Trypsin Neutralizing Solution (ATCC PCS-999-004)

References

References and other information relating to this material are available at www.atcc.org.

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The product is provided 'AS IS' and the viability of ATCC® products is warranted for 30 days from the date of shipment, provided that the customer has stored and handled the product according to the information included on the product information sheet, website, and Certificate of Analysis. For living cultures, ATCC lists the media formulation and reagents that have been found to be effective for the product. While other unspecified media and reagents may also produce satisfactory results, a change in the ATCC and/or depositor-recommended protocols may affect the recovery, growth, and/or function of the product. If an alternative medium formulation or reagent is used, the ATCC warranty for viability is no longer valid. Except as expressly set forth herein, no other warranties of any kind are provided, express or implied, including, but not limited to, any implied warranties of merchantability, fitness for a particular purpose, manufacture according to cGMP standards, typicality, safety, accuracy, and/or noninfringement.

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