Universal Neutralization Buffer

Catalog #: NB001

Product Size: 100 ml

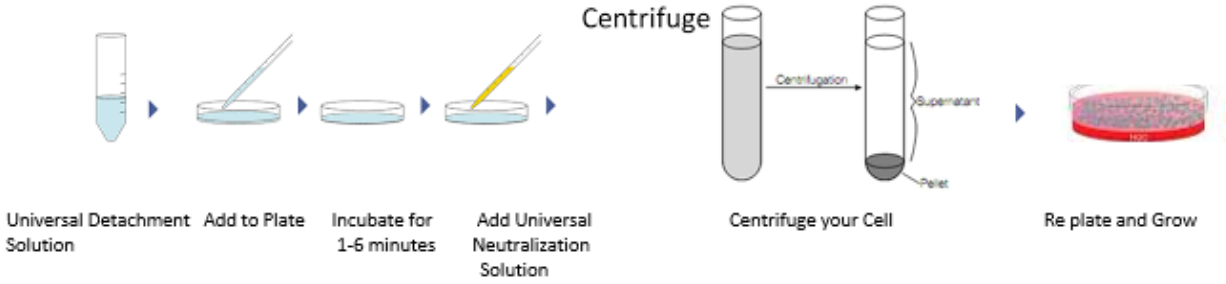
Storage: -20ºC until ready for culture.
While Culturing keep in 37°C CO_2 incubator

Product Format: Frozen

GENERAL INFORMATION

Universal Neutralization Buffer is a Universal Neutralization Buffer specifically formulated to rapidly inactivate the concentration of trypsin found in the Universal Detachment Solution (AD002) for Primary Cells solution.

Each type of cell or cell line responds to Universal Detachment Solution 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.



PROTOCOL

1. Bring the DPBS, the Universal Detachment Solution (AD002) for Primary Cells, and the Universal Neutralization Buffer to room temperature before use. Warm the complete growth medium to 37°C.

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.

3. Using 1 to 2 mL for every 25 cm2, add the appropriate volume of Universal Detachment Solution to each flask (e.g., each T-25 flask would be dissociated with 1 to 2 mL).

4. Gently rock each flask to ensure complete coverage of the Universal Detachment Solution over the cells, and then aspirate the excess fluid off 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 1 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.

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6. When most cells appear to have detached, quickly add an equal volume of the Universal Neutralization Buffer to each flask. Gently pipette or swirl the culture to ensure all the Universal Detachment 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 Universal Detachment Solution - 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 neutralized dissociation solution and re-suspend 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% CO2 incubator and incubate for at least 24 to 48 hours before processing the cells further.

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