Dispase (1 U/mL)

1 U/mL dispase in DMEM/F-12

Catalog # 07923 100 mL

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Product Description

Dispase is a protease that is suitable for the gentle dissociation of a wide variety of tissues. This product has been optimized for use in enzymatic passaging of human embryonic stem (ES) cells and human induced pluripotent stem (iPS) cells.

This product contains 1 U/mL Dispase II (neutral protease from Bacillus polymyxa) dissolved in DMEM/F-12.

Properties

Storage: Store at -20°C.
Shelf Life: Stable until expiry date (EXP) on label.

Handling / Directions For Use

NOTE: If product is received partially thawed, place immediately at -20°C or thaw and aliquot as described below. Do not use Dispase past the expiry date as indicated on the label.

Thaw Dispase at 2 - 8°C overnight. Once thawed, use immediately or store at 2 - 8°C for up to 2 weeks. Alternatively, aliquot and store at -20°C until expiry date as indicated on label. After thawing the aliquots, use immediately or store at 2 - 8°C for up to 2 weeks. Do not re-freeze.

Incubation with pre-warmed Dispase will dissociate cells with minimal cell damage from minced tissues or cultures on tissue culture plastic. Unlike trypsin, Dispase is not inhibited by serum. Dispase activity is inhibited by EDTA and EGTA. Dispase should be removed by dilution, for example, by washing cells with buffer or culture medium, or by centrifugation of cell suspensions.

PASSAGING HUMAN ES OR iPS CELLS
The following is a protocol for passaging human ES or iPS cells grown in mTeSR™1 (Catalog #85850) or TeSR™2 (Catalog #05860) on Corning® Matrigel® hESC-Qualified Matrix (Corning Catalog #354277). For complete instructions on culturing human ES and iPS cells, refer to the Technical Manuals: Maintenance of Human Pluripotent Stem Cells in mTeSR™1 (Document #28315) or TeSR™2 (Document #28210), available at www.stemcell.com or contact us to request a copy.

The procedure described below uses mTeSR™1 medium. If TeSR™2 is used instead, adjust the incubation time as indicated in step 5. Volumes are given for 6-well plates; if using other cultureware, adjust volumes according to surface area.

1. Aliquot sufficient mTeSR™1, Dispase, and DMEM/F-12 with 15 mM HEPES (Catalog #36254) for passaging cells and warm to room temperature (15 - 25°C).
2. Use a microscope to visually identify regions of differentiation. Mark these using a felt tip or lens marker on the bottom of the plate. NOTE: This selection should not exceed 20% of the well if the culture is of high quality.
3. Remove regions of differentiation by scraping with a pipette tip or by aspiration.
4. Aspirate medium from the well and rinse with 2 mL/well of DMEM/F-12.
5. Add 1 mL/well of Dispase and incubate at 37°C for 7 minutes if using mTeSR™1, or for 3 - 4 minutes if using TeSR™2. NOTE: After incubation the colony edges will appear slightly folded back but the colonies should remain attached to the plate.
6. Aspirate Dispase, and gently rinse each well 2 - 3 times with 2 mL/well of DMEM/F-12 to dilute away any remaining Dispase. NOTE: The incubation time may vary when using different cell lines, therefore dissociation should be monitored under the microscope until the optimal time is determined.

7. Add 2 mL/well of mTeSR™1. Gently detach colonies by scraping with a glass pipette or a cell scraper (e.g. Catalog #38065).
   NOTE: Take care to minimize the breakup of colonies.
8. Transfer the detached cell aggregates to a 15 mL conical tube (e.g. Catalog #38009).
9. Carefully pipette the cell aggregate mixture up and down 2 - 3 times with a 2 mL serological pipette (e.g. Catalog #38002) to break up the aggregates. Do not create a single-cell suspension.
10. Plate the cell aggregates with mTeSR™1 onto a new matrix-coated plate.
11. Place the plate in a 37°C incubator. Move the plate in several quick, short, back-and-forth and side-to-side motions to disperse cells across the surface of the well.
    NOTE: Ensure that newly seeded cell aggregates are evenly dispersed across the entire surface. Uneven distribution may result in differentiation of human ES or iPS cells.
12. Perform daily medium changes using mTeSR™1.