AssayComplete™ Freezing Reagent

Materials Provided

Catalog Number: 92-51XXFR Series
Quantity Shipped: 2 X 50 mL
Lot Number: See product label

Description

Ready-to-use AssayComplete Freezing Reagent has been optimized for consistent cryopreservation of DiscoverX cell lines to ensure optimal protection, high cell viability and maximal assay performance upon recovery from frozen storage.

Product Information

Storage Conditions: Store at -20°C. Thaw contents at room temperature and mix well by gently inverting the bottle prior to use. Once thawed, store at 4°C for up to 4 weeks. Avoid multiple freeze/thaw cycles.
Shelf Life: See product label for expiration date.
Shipping Conditions: Frozen on dry ice (-70°C).
Instructions on Use: Refer to cell line user manual.

Related Products*

AssayComplete Cell Culture Kits (92-31XXG Series)
AssayComplete Thawing Reagents (92-41XXTR Series)
AssayComplete Cell Plating Reagents (93-0563R Series)
AssayComplete Cell Detachment Reagent (92-0009)

*Refer to cell line datasheet to determine catalog numbers for each related product required for the assay.

Notice to Purchaser

AssayComplete Freezing Reagent has been optimized with DiscoverX cell-based assays. Occasionally, the reagent may be yellow or pink in color. This indicates a slight change in pH has occurred. It has been determined that using discolored media does not adversely impact cell growth or assay performance. Refer to the FAQ section for a more detailed explanation of this phenomenon.

For research use only. Not intended for use in diagnostic or therapeutic procedures.

Quality Control Data

AssayComplete Freezing Reagent is performance tested on DiscoverX cell lines. Each lot of AssayComplete Freezing Reagent is subjected to standard evaluation tests for the absence of bacterial, fungal and mycoplasma contaminants.

This product includes high quality serum that has been tested for optimal performance. Testing includes heat inactivation, sterility, endotoxin (≤5 EU/ml), pH (≥6.9 to ≤7.8), osmolality (≥280 to ≤340), total protein (≥3.0 to ≤5.0), hemoglobin (≤10 mg/dl), mycoplasma, virus, and bacteriophage levels determination.

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Instructions for Use

The following is a procedure for freezing cells that have been cultured in T75 or T225 flasks. This protocol assumes that cells have reached 70% to 80% confluency in enough flasks to fill the desired number of cryovials, in 1 mL/vial aliquots, with the desired number of cells per vial (e.g. 1 X 10^6 per vial).

Cell Freezing

Keep cells on ice during this process to protect cell viability.

1. Remove T75 (or T225) flasks from incubator and place in a sterile tissue culture hood.
2. Gently aspirate the media from the flasks.
3. Add 10 mL PBS into each T75 flask (or 15 mL for a T225 flask), and swirl to rinse the cells.

Care should be taken in handling to avoid contamination.

4. Gently aspirate PBS from the flask.
5. Add 1 mL of 0.25% Trypsin-EDTA to T75 flasks (or 3 mL to T225 flasks).
6. Gently rock the flask back and forth to ensure the surface of the flask is thoroughly covered with Trypsin-EDTA.

Keep cells on ice during this process and transfer to a cryogenic container.

7. Incubate the flasks at 37°C, 5% CO_2 for 2 to 3 minutes or until the cells have detached.
8. Remove the flask from the incubator and view under a microscope to confirm that the cells have detached. If necessary, tap the edge of the flask to detach cells from the surface.
9. Add 5 mL AssayComplete™ Cell Culture Reagent to each T75 flask (or 15 mL to each T225 flask).
10. Using a pipette, gently rinse the cells from the surface of the flask with the added media. Gently pipette up and down several times to achieve a single cell suspension with no cell clumps.
11. Remove the entire amount of cells from the T75 flask and transfer to a 15 mL conical centrifuge tube. If using a T225 flask, transfer cells to a 50 mL conical centrifuge tube. If necessary, add additional AssayComplete Cell Culture Reagent to the flasks (e.g. 3 mL for T75 flasks or 5 mL for T225 flasks), rinse to collect the remaining cells, then transfer the additional media to the conical tube. Gently pipette up and down several times to ensure a single cell suspension.
12. For the purpose of determining the concentration of cells in the suspension:
    a. Set aside a small fraction (0.5 mL or less) of the suspended cells in a separate tube.
    b. Transfer an appropriate portion of this fraction to a hemocytometer (typically 10 µL of cell suspension) or another cell counting device.
    c. Count cells, calculate the concentration of cells in the suspension, and then calculate the total number of cells remaining in the 15 mL (or 50 mL) centrifuge tube.
13. Centrifuge the collected cells at 300 X g for 4 minutes.
14. After centrifugation, discard the supernatant being careful not to disturb the cell pellet.
15. Based on the total cell number calculated in step 12 above, re-suspend cells to the desired concentration (e.g. 1 X 10^6 to 2 X 10^6 cells/mL) with ice cold AssayComplete Freezing Reagent.
16. Make 1 mL aliquots by transferring 1 mL of cell suspension to labeled 2 mL cryogenic tubes; tightly cap the tubes.

Keep cells on ice during this process to protect cell viability.

17. Freeze cells in a –80°C freezer at a controlled rate (-1°C/minute) overnight. This can be performed using a dedicated cell freezer or commercially available freezing chamber (pre-chilled to 4°C). For short term storage, vials can be stored in the -80°C freezer for a maximum of two weeks.

FAQ

After the reagent was thawed, the media was discolored, is this normal?

Variations in color of the media reagent can occur. These variations result from CO_2 vapor from the dry ice in the shipping package altering the concentrations of dissolved CO_2 in the media reagents. This can result in slight changes of pH that will induce a yellowing of the phenol red indicator dye present in the media.

DiscoverX has determined that use of media with a yellow color does not affect cell growth or assay performance. Exposure of discolored media to 5% CO_2 in a tissue culture incubator will re-balance the media to proper physiological pH which will normalize the color accordingly.

For additional information or Technical Support, see contact information below.

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