# AssayComplete™ Thawing Reagent

## Materials Provided

**Catalog Number:** 92-41XXTR Series
**Quantity Shipped:** 100 mL
**Lot Number:** See product label

## Description

AssayComplete Thawing Reagents have been optimized to revive cells from a frozen state to ensure high post-thaw viability, high yield recovery, improved cell plating efficiency, and maximal assay performance for cryopreserved DiscoverX cell lines.

## Product Information

**Storage Conditions:** Store at -20°C. Thaw contents at 37°C 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 Freezing Reagents (92-51XXFR 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 Thawing 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 Thawing Reagents are performance tested on DiscoverX cell lines. Each lot of AssayComplete Thawing 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.

## Instructions for Use

The following procedures are for thawing cells in cryovials and propagating them in a T75 flask.

### Cell Thawing

The following is a protocol for thawing cells in a T75 flask.

Care should be taken in cell handling to avoid contamination.

1. Pre-warm AssayComplete™ Thawing Reagent in a 37°C water bath for 15 minutes.
2. Add 15 mL of the AssayComplete Thawing Reagent into a T75 flask in a sterile tissue culture hood. Set aside for step 6 below.

Safety Warning: When removing cryovials from liquid nitrogen storage, use tongs and place immediately on dry ice in a covered container. Wait at least 1 minute for any liquid nitrogen inside the vial to evaporate. Do not touch the bottom of the vials at any time to avoid inadvertent thawing of the cells.

3. Remove the cell cryovials from -80°C or liquid nitrogen vapor storage and immediately place them on dry ice prior to thawing.
4. Place the frozen cell vials briefly (30 seconds to 1 minute) in a 37°C water bath, under sterile conditions, until only small ice crystals remain and the cell pellet is almost completely thawed.
5. Decontaminate the vial by spraying and wiping with 70% ethanol, and transfer it to a tissue culture hood.
6. With a pipette, gently transfer the thawed cells to the pre-filled T75 flask and incubate at 37°C, 5% $CO_2$.
7. Maintain the cells in culture until they are >70% confluent. Then proceed to “Cell Propagation” instructions. Do not split if cells are below this confluency or growth issues may occur.

### Cell Propagation

The following is a protocol for propagating cells once they become ≥70% confluent in a T75 flask.

1. Pre-warm AssayComplete Thawing reagent in a 37°C water bath for 15 minutes.
2. Remove the T75 flask from the tissue culture incubator and place in a sterile tissue culture hood.
3. Gently aspirate media from the T75 flask.
4. Add 5 mL PBS into the T75 flask, very gently tip the flask side to side allowing PBS to cover the entire face of the flask to rinse the cells.
5. Gently aspirate PBS from flask.
6. Add 1 mL of 0.25% Trypsin-EDTA to the T75 flask.
7. Gently rock the flask back and forth to ensure the surface of the flask is thoroughly covered with trypsin.
8. Incubate the flask at 37°C and 5% $CO_2$ for 2 to 3 minutes or until the cells have detached.
9. Remove the flask from the incubator and confirm the cells are detached by viewing under a microscope. If necessary, gently tap the edge of the flask to detach cells from the surface. If the cells do not detach, return to the incubator for an additional 1 to 2 minutes and repeat this step until cells are in suspension.

Excessive treatment with Trypsin- EDTA may compromise cell viability.

10. Add 4 mL of AssayComplete Thawing reagent to the T75 flask.
11. Using a pipette, gently rinse the cells from the surface of the flask with the added media.
12. Split the cells conservatively for the first passage after thaw using AssayComplete™ Thawing reagent. Typical (conservative) split ratios for common cell backgrounds are included in the table below:

| Cell Background | Suggested Split |
|---|---|
| CHO-K1 | 1:5 |
| HEK 293 | 1:3 |
| U2OS | 1:2 |

For example, for CHO-K1 cells, add 4 mL of AssayComplete Thawing reagent to the flask containing 1 mL of 0.25% Trypsin-EDTA, then transfer 1 mL (1/5th of the total reagent in the flask) into each new tissue culture flask.

13. Fill a new T75 or T225 flask with AssayComplete Thawing Reagent until the total volume equals 12 mL for T75 flasks (or 25 mL for T225 flasks), and add cell suspension (added volume determined at step 12) to the media in the flask. Transfer flask to a tissue culture incubator and incubate cells for 24 hours at 37°C, 5% $CO_2$.
14. After 24 hours, examine the cells under a microscope. If the cells appear to be healthy (adhering to the surface of the flask with only a small number of cells remaining in suspension) exchange the AssayComplete Thawing Reagent with AssayComplete Cell Culture Reagent; 12 mL for T75 flasks (or 25 mL for T225 flasks) (Refer to cell line datasheet to determine the correct Cell Culture Kit for your cell line). Then return the flask to a tissue culture incubator. If the cells do not appear to be healthy or if confluency is <25%, incubate for an additional 24 to 48 hours to allow for additional cell recovery before executing this step.
15. Once the cells become ≥70% confluent in the flask, split the cells every 2 to 3 days, based on the doubling time of the cell line, using AssayComplete Cell Culture Reagent. Typical split ratios for common cell backgrounds are included in the table below:

| Cell Background | Suggested Split Ratio |
|---|---|
| CHO-K1 | 1:10 |
| HEK 293 | 1:5 |
| U2OS | 1:3 |

To maintain logarithmic growth of the cells, cultures should be maintained in a sub-confluent monolayer.

Do not use trypsin if detaching cells in preparation for setting up an assay. Refer to Cell Preparation and Plating protocol in cell line user manuals for detailed instruction on handling cells for assay preparation.

## 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.