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# StemPro® Accutase® Cell Dissociation Reagent

## Description

StemPro® Accutase® Cell Dissociation Reagent is a ready-to-use cell detachment solution of proteolytic and collagenolytic enzymes. StemPro® Accutase® does not contain mammalian or bacterial derived products and is useful for the routine detachment of cells from standard tissue culture plasticware and adhesion coated plasticware including Geltrex® and CELLStart™ polymers. StemPro® Accutase® can be substituted directly for Trypsin in cell dissociation protocols without the need for inactivation reagents. StemPro® Accutase® outperforms enzymes of animal origin such as Trypsin, maintaining higher cell viability following detachment of primary and stem cells. Some downstream applications following Accutase® treatment include analysis of cell surface markers, virus growth assay, cell proliferation, tumor cell migration assays, routine cell passage, production scale-up (bioreactor), and flow cytometry. Cell lines tested for Accutase® application include human embryonic stem cells, human mesenchymal stem cells, human neural stem cells, and primary cells including macrophages, fibroblasts, keratinocytes, vascular endothelial cells, hepatocytes, and established cell lines such as adherent CHO, BHK, and 293 cells.

| Product | Catalog no. | Amount | Storage | Shelf life* |
|---|---|---|---|---|
| StemPro® Accutase® Cell Dissociation Reagent | A11105-01 | 100 mL | –20°C to –5°C; Protect from light | 24 months |

*Shelf life duration is determined from Date of Manufacture.

## Product use

For Research Use Only. Not for use in diagnostic procedures.

## Important information

- Thaw StemPro® Accutase® at 2°C to 8°C overnight or at room temperature with regular gentle swirling to ensure thermal homogeneity. **Do not** thaw at 37°C.
- **Do not** store StemPro® Accutase® at room temperature.
- Store StemPro® Accutase® in the dark at 2°C to 8°C once thawed. **Do not** refreeze; StemPro® Accutase® is stable up to 2 years within its expiration date when stored as directed.
- StemPro® Accutase® does not require use of an inactivation reagent or serum-supplemented media. StemPro® Accutase® is inactivated by dilution alone.

## Safety information

For every chemical, read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

## Use

StemPro® Accutase® substitutes directly, into existing cell dissociation protocols, as an alternative to trypsin. Cell lines tested for StemPro® Accutase® application include but are not limited to: human embryonic stem cells, human mesenchymal stem cells and human neural stem cells; fibroblasts, keratinocytes, vascular endothelial cells, hepatocytes, vascular smooth muscle cells, hepatocyte progenitors, primary chick embryo neuronal cells, bone marrow stem cells, adherent CHO and BHK cells, macrophages, 293 cells, L929 cells, immortalized mouse testicular germ cells, 3T3, Vero, COS, HeLa, NT2, MG63, M24 and A375 metastatic melanoma, gliomas U251, D54, HT1080 fibrosarcoma cells, and Sf9 insect cells.

**Note:** The following procedures are designed to dissociate cells on 60-mm dish. Adjust volumes accordingly for alternate vessel sizes.

## General dissociation

1. Aspirate the medium and wash with 4 mL of DPBS without calcium and magnesium.
2. Add StemPro® Accutase® to culture dish or flask using aseptic procedures at 2 mL per 60-mm dish (10 mL per 75 $cm^2$ surface area).
3. Return culture to 37°C incubator and allow cells to detach 1–10 minutes.
4. Determine viable cell density using a Countess® Automated Cell Counter (or similar automated or manual method) and passage as usual; no additional washes or enzyme inhibitors are required.

## Dissociation of cells grown on Geltrex® hESC-qualified or CELLStart™-coated dishes

This protocol is appropriate for the subculture of human ESCs grown in StemPro® hESC SFM and human or rat NSCs grown in StemPro® NSC SFM.

1. Aspirate medium from culture dish and wash with 4 mL of DPBS without calcium and magnesium.
2. Aspirate DPBS and add 2 mL of StemPro® Accutase® to culture dish.
3. Incubate for 2–5 minutes at 37°C until individual single cells start to round up.
4. Gently pipet StemPro® Accutase® solution across plate surface to remove cells off the plate surface.
5. Transfer cell suspension to a sterile 15-mL conical tube. Gently pipet up and down until cells are dispersed into a single cell suspension.
6. Add 8 mL of appropriate pre-warmed medium to rinse any remaining cells off the dish's surface and transfer to the conical tube (*from step 5*).
7. Take a 20 uL sample of the cell suspension to determine viable cell density using a Countess® Automated Cell Counter.
8. Centrifuge the cell suspension at 200 × *g* for 4 minutes. Ascertain presence of cell pellet.

9. Aspirate supernatant and resuspend the cell pellet in 1 mL fresh pre-warmed complete medium. Inoculate pre-coated 60-mm culture dishes containing 5 mL pre-warmed complete medium, with predetermined volume of cell suspension (see following note for appropriate plating densities).

   **Note:** Plate hESCs at $0.5 \times 10^6$ to $1 \times 10^6$ cells/60-mm dish in StemPro® hESC SFM. Plate human and rat NSCs at $1 \times 10^6$ cells/60-mm dish in Stempro® NSC SFM.

10. Return cells to incubator at 37°C with a humidified atmosphere of 5% $CO_2$ in air.

### Dissociation of human or rat neurosphere suspension cultures grown in StemPro® NSC SFM

1. Transfer neurosphere cell suspension from culture dish to a sterile 15-mL conical tube.
2. Allow neurospheres to settle to the bottom of the tube (~2–5 minutes) before proceeding to Step 3. Alternatively, the neurospheres can be centrifuged at 200 × *g* for 2 minutes.
3. Carefully aspirate medium leaving the neurospheres at the bottom of tube in a minimal volume (~100 µL) of remaining media.
4. Resuspend neurospheres in 5 mL DPBS without calcium and magnesium.
5. Repeat steps 2 and 3 leaving the neurospheres at the bottom of tube in a minimal volume (~100 µL) of DPBS.
6. Add 1 mL of StemPro® Accutase® to the neurospheres and incubate 10 minutes at room temperature.
7. Gently pipet up and down using a wide bore pipette tip (i.e., 1000 µL), until all the neurospheres are dispersed into a single cell suspension.
8. Add 4 mL of fresh pre-warmed complete medium to the cell suspension.
9. Centrifuge the cell suspension at 200 × *g* for 4 minutes.
10. Carefully aspirate the supernatant and resuspend the cell pellet in 1 mL fresh pre-warmed complete medium.
11. Determine viable cell density of a 20 uL sample using a Countess® Automated Cell Counter.
12. Dilute the cell suspension with pre-warmed complete medium to $2 \times 10^5$ viable cells/mL. Dispense into new 60-mm culture dishes (5 mL/dish) and incubate at 37°C in a humidified atmosphere of 5% $CO_2$ in air.
13. Refeed neurosphere cultures every 2–3 days by following steps 1–3 and resuspending in 5 mL fresh pre-warmed complete medium. Passage cells when neurospheres reach ≥3.5 mm in diameter.

### Related products

| Product | Catalog no. |
|---|---|
| Dulbecco's Phosphate Buffered Saline, without calcium and magnesium | 14190 |
| Distilled Water | 15230 |
| TrypLE™ Express (1X), liquid, without Phenol Red | 12604 |
| StemPro® NSC SFM | A10509-01 |
| StemPro® hESC SFM | A10007-01 |
| Geltrex® LDEV-Free hESC-qualified Reduced Growth Factor Basement Membrane Matrix | A14133 |
| Geltrex® LDEV-Free Reduced Growth Factor Basement Membrane Matrix | A14132 |
| CELLStart™ CTS™ | A10142 |
| Trypan Blue Solution, 0.4% | 15250 |
| Countess® Automated Cell Counter | C10227 |

### Explanation of symbols and warnings

The symbols present on the product label are explained below:

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|  |  |  |  |  |
| Batch code | Manufacturer | Catalog number | Keep away from light | Use by |

| | | | |
|---|---|---|---|
|  |  |  |  |
| Caution, consult accompanying documents | Consult instructions for use | Temperature limitation | Sterilized using aseptic processing techniques |

### Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

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For further assistance, email **techsupport@lifetech.com**