

StemPro™ Accutase™ Cell Dissociation Reagent

Catalog Number A1110501

Pub. No. MAN0007245 Rev. B



WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from [thermofisher.com/support](https://www.thermofisher.com/support).

Product information

StemPro™ Accutase™ Cell Dissociation Reagent is a ready-to-use cell detachment solution of proteolytic and collagenolytic enzymes. StemPro™ Accutase™ Cell Dissociation Reagent 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™ matrix. StemPro™ Accutase™ Cell Dissociation Reagent can be substituted directly for Trypsin in cell dissociation protocols without the need for inactivation reagents. StemPro™ Accutase™ Cell Dissociation Reagent outperforms enzymes of animal origin such as Trypsin, maintaining higher cell viability following detachment of primary and stem cells. Some downstream applications following StemPro™ Accutase™ treatment include analysis of cell surface markers, virus growth assays, cell proliferation, tumor cell migration assays, routine cell passage, production scale-up (bioreactor), and flow cytometry. Cell lines tested for StemPro™ 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.

Contents and storage

Component	Cat. No.	Amount	Storage	Shelf life ^[1]
StemPro™ Accutase™ Cell Dissociation Reagent	A1110501	100 mL	-20°C to -5°C; Protect from light.	24 months

^[1] Shelf-life duration is determined from the Date of Manufacture.

Procedural guidelines

- Thaw StemPro™ Accutase™ Cell Dissociation Reagent 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™ Cell Dissociation Reagent at room temperature.
- Store StemPro™ Accutase™ Cell Dissociation Reagent in the dark at 2°C to 8°C once thawed. Do not refreeze. StemPro™ Accutase™ Cell Dissociation Reagent is stable up to 2 years within its expiration date when stored as directed.
- StemPro™ Accutase™ Cell Dissociation Reagent does not require use of an inactivation reagent or serum-supplemented media. StemPro™ Accutase™ Cell Dissociation Reagent is inactivated by dilution alone.

Use

StemPro™ Accutase™ Cell Dissociation Reagent substitutes directly, into existing cell dissociation protocols, as an alternative to trypsin. Cell lines tested for StemPro™ Accutase™ Cell Dissociation Reagent 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™ Cell Dissociation Reagent to culture dish or flask using aseptic procedures at 2 mL per 60-mm dish (10 mL per 75 cm² 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-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™ Cell Dissociation Reagent 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™ Cell Dissociation Reagent 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 µL 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 0.5×10^6 to 1×10^6 cells/60-mm dish in StemPro™ hESC SFM. Plate human and rat NSCs at 1×10^6 cells/60-mm dish in StemPro™ NSC SFM.
10. Return cells to incubator at 37°C with a humidified atmosphere of 5% CO₂ 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). 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™ Cell Dissociation Reagent 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 µL sample using a Countess™ Automated Cell Counter.
12. Dilute the cell suspension with pre-warmed complete medium to 2×10^5 viable cells/mL. Dispense into new 60-mm culture dishes (5 mL/dish) and incubate at 37°C in a humidified atmosphere of 5% CO₂ 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

Unless otherwise indicated, all materials are available through thermofisher.com.

Catalog numbers that appear as links open the web pages for those products.

Product	Catalog no.
DPBS, no calcium, no magnesium	14190144
TrypLE™ Express Enzyme (1X), no phenol red	12604021
StemPro™ NSC SFM	A1050901
StemPro™ hESC SFM	A1000701
Geltrex™ LDEV-Free, hESC-Qualified, Reduced Growth Factor Basement Membrane Matrix	A1413301
Geltrex™ LDEV-Free Reduced Growth Factor Basement Membrane Matrix	A1413202
Trypan Blue Solution, 0.4%	15250061
Countess™ 3 Automated Cell Counter	AMQAX2000

Limited product warranty

Life Technologies Corporation and its affiliates warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at www.thermofisher.com/us/en/home/global/terms-and-conditions.html. If you have questions, contact Life Technologies at www.thermofisher.com/support.



Life Technologies Corporation | 3175 Staley Road | Grand Island, New York 14072 USA

For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: Pub. No. MAN0007245 B

Revision	Date	Description
B	20 December 2024	Updated branding. Changed trademark symbol. The version numbering was changed from a numerical format to a letter-based format in accordance with internal document control procedures.
1.0	12 August 2014	New document for StemPro™ Accutase™ Cell Dissociation Reagent.

The information in this guide is subject to change without notice.

DISCLAIMER: TO THE EXTENT ALLOWED BY LAW, THERMO FISHER SCIENTIFIC INC. AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE, OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

Important Licensing Information: This product may be covered by one or more Limited Use Label Licenses. By use of this product, you accept the terms and conditions of all applicable Limited Use Label Licenses.

©2014-2024 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified.