

Cell Dissociation Reagent

StemPro Accutase Cell Dissociation Reagent is a ready-to-use cell detachment solution of proteolytic and collagenolytic enzymes. Useful for the routine detachment of cells from standard tissue culture plasticware and adhesion coated plasticware, including Geltrex™ Reduced Growth Factor Basement Membrane Matrix, CELLStart™ and polymers. Accutase performs exceptionally well in detaching cells for the analysis of cell surface markers, virus growth assay, quiescence assays by serum starvation, transformation assays by oncogene transfection, neural crest cell migration assays, cell proliferation, cell haptotaxis, tumor cell migration assays routine cell passage, production scale-up (bioreactor), and flow cytometry. Cell lines tested for Accutase application includes 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, Sf9 insect cells, human embryonic stem cells, human mesenchymal stem cells and human neural stem cells. Accutase does not contain mammalian or bacterial derived products.

Description	Cat. No.	Size
StemPro Accutase Cell Dissociation Reagent	A11105-01	1 x 100mL

Intended Use

For research use only. CAUTION: Not intended for human or animal diagnostic or therapeutic uses.

Precautions

Do not store Accutase at room temperature. Accutase is stable when stored at 2 to 8°C up to 2 years.

It is recommended to thaw Accutase at room temperature or 4°C overnight. **Do not thaw at 37°C.**

Storage

Store at -5 to -20°C, Protect from light.

Shelf Life

24 months

Use:

Note: The following procedures are designed to dissociate cells on 60mm dish. Volumes should be adjusted accordingly for desired vessel size.

General Dissociation:

- Aspirate the medium and wash with 4 mL of DPBS (w/o calcium and magnesium, Cat. No 14190).
- Add Accutase to culture dish or flask using aseptic procedures at 2 mL per 60mm surface area (10 mL per 75cm² surface area).
- Return culture to 37°C incubator and allow cells to detach 5 to 10 minutes.
- Count cells and passage as usual; no additional washes or enzyme inhibitors are required.

Dissociation of human ESCs grown in StemPro hESC SFM on Geltrex hESC-qualified or CELLstart coated dishes:

- Aspirate the medium from culture dish and wash with 4 mL of DPBS (w/o calcium and magnesium, Cat. No 14190).

- Aspirate DPBS and add 2 mL of Accutase to culture dish.
- Incubate for 2 to 5 minutes at 37°C until individual single cells start to round up.
- Gently rinse to remove cells off of the plate's surface.
- Transfer cell suspension to 15 mL conical tube. Gently pipette up and down until cells are in a single cell suspension.
- Add 8 mL of medium to rinse any remaining cells off of the dish's surface and transfer to the conical tube (from Step 5).
- Take a 20 uL sample of the cell suspension to determine viable cell density.
- Centrifuge conical tube containing the cell suspension at 200g for 4 minutes.
- Aspirate supernatant, resuspend in fresh medium and plate on coated dish(s). Incubate at 36 to 38°C in a humidified atmosphere of 5% CO₂ in air.

Note: Plating efficiency of 0.5-1x10⁶ cells/60mm dish is optimal for the culture system of StemPro hESC SFM with Geltrex.

Dissociation of adherent human or rat NSCs grown in StemPro NSC SFM on CELLstart coated dishes:

- Aspirate the medium from culture dish and wash with 4 mL of DPBS (w/o calcium and magnesium, Cat. No 14190).
- Aspirate DPBS and add 2 mL of Accutase to culture dish.
- Incubate for 2 to 5 minutes at 37°C until individual single cells start to round up.
- Gently rinse to remove cells off of the plate's surface.
- Transfer cell suspension to 15 mL conical tube. Gently pipette up and down until cells are in a single cell suspension.
- Add 8 mL of medium to rinse any remaining cells off of the dish's surface and transfer to the conical tube (from Step 5).
- Take a 20 uL sample of the cell suspension to determine viable cell density.
- Centrifuge conical tube containing the cell suspension at 200g for 4 minutes.

- Aspirate supernatant, resuspend in fresh medium and plate on coated dish(s). Incubate at 36 to 38°C in a humidified atmosphere of 4 to 6% CO₂ in air.

Note: Plating efficiency of 1x10⁶ cells/60mm dish is optimal for the culture system of StemPro NSC SFM with CellStart.

Dissociation of human or rat neurosphere cultures grown in StemPro NSC SFM:

- Remove neurosphere cell suspension from culture dish and transfer to a 15 mL conical tube.
- Let neurospheres settle down in the tube (~2 to 5 minutes) before proceeding to Step 3. Alternatively, the cells can be centrifuged at 100g for 1 minute.
- Gently aspirate medium leaving the neurospheres at the bottom of tube with approximately 100µL of media remaining.
- Resuspend neurospheres in 5 mL DPBS (w/o calcium and magnesium, Cat. No 14190).
- Let neurospheres settle down in the tube (2 to 5 minutes) before proceeding to Step 6. Alternatively, the cells can be centrifuged at 100g for 1 minute.
- Gently aspirate DPBS leaving the neurospheres at the bottom of tube with approximately 100µL of DPBS remaining.
- Add 1 mL of Accutase to the neurospheres and incubate 10 minutes at room temperature.
- Using the proper sized pipette tip (i.e. 1000µl), pipette up and down until all the neurospheres are in a single cell suspension.
- Add 4 mL of fresh medium to the tube.
- Centrifuge the cells at 200g for 4 minutes.
- Gently aspirate the supernatant.
- Resuspend cells in fresh medium, transfer to a new culture dish and incubate at 36 to 38°C in a humidified atmosphere of 4 to 6% CO₂ in air.

Note: 200,000 cells/mL of cell density can be used for cells in Stempro NSC SFM

Related Products

StemPro hESC SFM (A1000701)

StemPro NSC SFM (A1050901)

Geltrex hESC-qualified (A10480)

CELLstart (A10142)

FGF – basic (50ug/mL) (PHG0026)

2-Mercaptoethanol (21985)

Dulbecco's Phosphate Buffered Saline (DPBS) without calcium, magnesium, or phenol red (1X), liquid (14190)

Dulbecco's Phosphate Buffered Saline (DPBS) with calcium, and magnesium (1X), liquid (14040)

GLUTAMAX™ -I, 200mM (100X), liquid (35050)

Technical Support

For additional product and technical information, such as Material Safety Data Sheets (MSDS), Certificate of Analysis, etc, please visit our website at www.invitrogen.com. For further assistance, please email our Technical Support team at Techsupport@Invitrogen.com.

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