



Stem Cells

Handle with care: gentle dissociation product for sensitive cells

StemPro® Accutase® Cell Dissociation Reagent



- Efficient—gently detach adherent cells within minutes
- Convenient and cost-effective—stable when frozen, refrigerated, and at room temperature
- Maximum viability—standardized protocols for human embryonic and neural stem cell lines

Gently dissociate your cells

At last, a gentler alternative to trypsin/EDTA. Accutase® reagent replaces this traditional but harsh reagent for the detachment and dissociation of anchorage-dependent cells and also reduces suspension cell clumping in preparation for counting. In single-cell disaggregation, trypsin and other agents cause extremely low cell viability.¹ The advantages of Accutase® over traditional trypsin/EDTA treatment include less damage to cells, increased viability, and lower risk of introducing adventitious agents into cell cultures because it does not contain any mammalian or bacterially derived proteins.

StemPro® Accutase® Cell Dissociation Reagent is a solution of proteolytic and collagenolytic enzymes. It is useful for routine detachment of cells from standard tissue culture and adhesion-coated plasticware, including Geltrex™ and CELLstart™ products, and polymers. Accutase® is exceptional at detaching cells for analyzing cell surface markers, virus growth assays, quiescence assays by serum starvation, transformation assays by oncogene transfection, neural crest cell migration assays, as well as cell proliferation, haptotaxis, and tumor cell migration assays, routine cell passage, production scale-up, and flow cytometry.

Versatile and maximizes cell viability

StemPro® Accutase® Cell Dissociation Reagent is a 1X ready-to-use reagent that dissociates a variety of primary and stem cells following expansion in culture using standard methods. These include FBS-supplemented media with or without feeder cell layers, and serum-free or xenofree media with feeders or feeder-free systems requiring cell attachment substrates such as Geltrex™ hESC-Qualified and CELLstart™ substrate. No dilution is required prior to cell dissociation with StemPro® Accutase® nor is protein needed to stop enzyme activity. StemPro® Accutase® is very gentle on cells and maintains high cell viability (Figure 1).

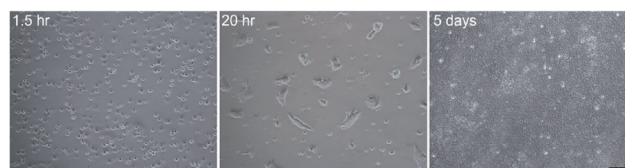


Figure 1—Robust single-cell passaging of hESCs. BG02 cells were disaggregated to single cells with Accutase® reagent and plated in StemPro® hESC SFM on a Geltrex™ substrate. Images taken 1.5 and 20 hours after plating indicate that cells migrate to form microcolonies. These cultures expand to form monolayers of undifferentiated cells after 5–6 days of growth.



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Consistent and stable lot performance

All Accutase® batches are tested for enzyme activity. A newly developed chromogenic assay that correlates with cell detachment efficiency was tested on every batch. The positive control was an Accutase® lot formulated in March 2006 and stored at 4°C. Figure 2 shows some of the positive control data points. In addition, values for “on-test” Accutase® lots illustrate formulation performance. Based on this data, the long-term stability of Accutase® is at least 2 years at 4°C (Figure 2).

Use with human embryonic and neural stem cell lines

StemPro® Accutase® testing in multiple human embryonic stem cell (hESC) and neural stem cell (NSC) lines show high viability, normal morphology, and equivalent gene expression markers (Figures 1 and 3). This reagent can also dissociate suspension cultures such as neural stem cell spheroids, embryoid bodies, and floating sphere colonies (Figure 4). Dissociation of human NSCs on CELLstart™ in StemPro® NSC SFM shows a mean viability of 90% after dissociation with StemPro® Accutase® reagent.

Learn more about StemPro® Accutase® at

www.invitrogen.com/accutase.

Reference

1. Amit, M. et al. (2000) Clonally derived human embryonic stem cell lines maintain pluripotency and proliferative potential for prolonged periods of culture. *Dev Biol* 227:271–278.

Ordering information

| Product | Quantity | Cat. no. |
|--|----------|-----------|
| StemPro® Accutase® Cell Dissociation Reagent | 100 mL | A11105-01 |

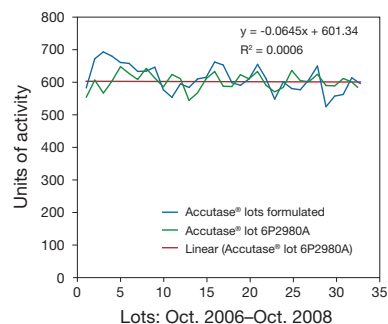


Figure 2—Accutase® long-term stability. Data show stability of at least two years at 4°C using a chromogenic assay as well as multiple-lot consistency.

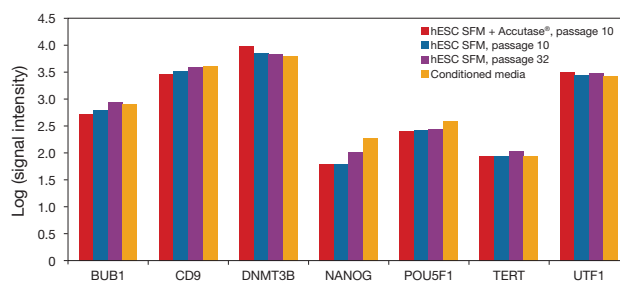


Figure 3—Microarray data of BG02 cells. hESCs were passaged using StemPro® Accutase® reagent in StemPro® hESC SFM and after 10 passages, pluripotent gene expression was compared to that of cells cultured to passage 10 using StemPro® hESC SFM and Collagenase Type IV, to passage 32 using StemPro® hESC SFM and Collagenase Type IV, and to passage 1 in KnockOut™ SR supplemented with MEF-conditioned medium.

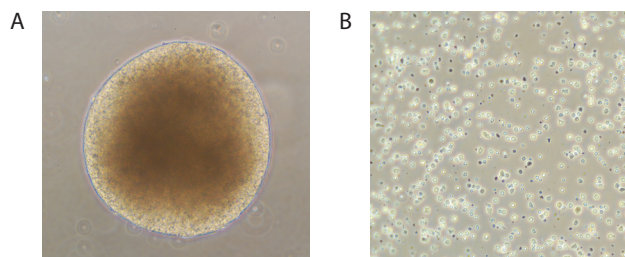


Figure 4—Dissociation of human neurosphere. Human neurosphere (A) dissociated into single-cell culture and (B) using 1 mL of StemPro® Accutase® reagent for 10 minutes.