Technical Data Sheet

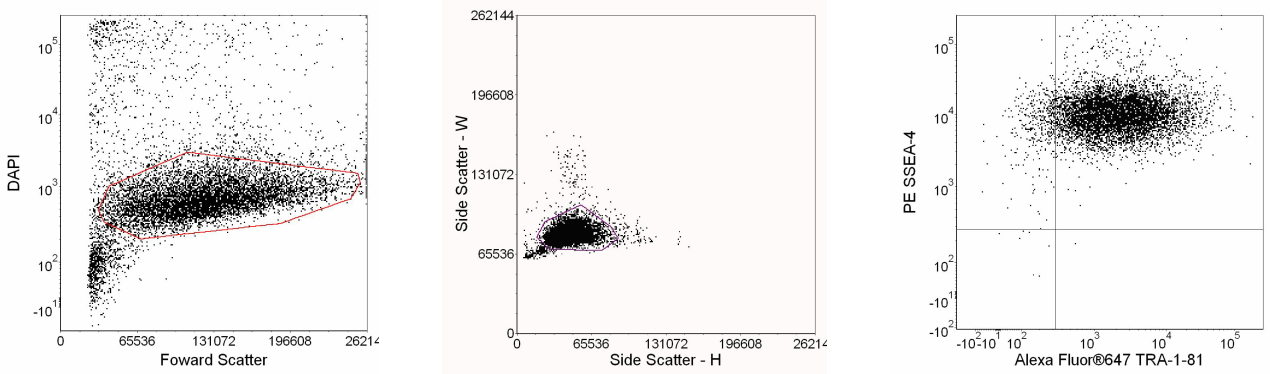
Accutase™ Cell Detachment Solution

Product Information

Material Number:	**561527**
Size:	100 ml
Storage Buffer:	Frozen, sterile aqueous buffered solution containing proprietary ingredients, EDTA, Phenol Red, and no preservative.

Description

Accutase™ is a cell detachment solution comprised of collagenolytic and proteolytic enzymes and does not contain mammalian or bacterial derived products. Accutase™ is a replacement for trypsin solution and can be used for the passaging of cell lines. Additionally, Accutase™ performs well when detaching cells for the analysis of many cell surface markers using flow cytometry and for cell sorting. Accutase™ has been demonstrated effective in detaching multiple cell types including: human and mouse embryonic and induced pluripotent stem cells, human neural stem cells, fibroblasts, keratinocytes, vascular endothelial cells, hepatocytes, neural cell types, bone marrow derived 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 and D54, HT1080 fibrosarcoma cells, and Sf9 insect cells.



Human Embryonic Stem (ES) cells detached with Accutase™ and analyzed with cell surface markers of pluripotency. *H9 ES cells (WiCell, Madison, WI) that were grown on BD Matrigel™ hESC-qualified Matrix (Cat. No. 354277) in mTeSR®1 medium (Stem Cell Technologies) were detached with Accutase™ Cell Detachment Solution. The cells were stained with either PE Mouse anti-SSEA-4 (Cat. 560128) and Alexa Fluor® 647 Mouse anti-Human TRA-1-81 (Cat. 560793) or PE Mouse IgG3, κ Isotype Control (Cat. 556659) and Alexa Fluor® 647 Mouse IgM, κ Isotype Control (Cat. 560806) and then analyzed with Diamidino-2-phenylindole dihydrochloride (DAPI) (Sigma, Cat. No. D-9542) for live/dead cell discrimination. Flow cytometry was performed on a BD LSR™ II flow cytometry system. For data analysis, live cells were first gated (left panel), and then single cells were selected by light scatter gating (center panel). Expression of surface pluripotency markers was then determined (right panel), with the positions of the quadrant markers based upon the isotype controls (data not shown).*

Preparation and Storage

The product should be kept undiluted at -20°C for long term storage, and it may be kept undiluted at 4°C for short term storage.

This product is shipped frozen, but may thaw during transit. On receipt, if it remains cool to the touch, it may be stored at 4 °C for up to 2 months or re-frozen and stored at -20 °C for up 2 years.

Application Notes

Application

Cell culture	Routinely Tested
Flow cytometry	Tested During Development

Recommended Assay Procedure:

To obtain a single-cell suspension: (Use aseptic techniques if you plan on continuing culture of cells.**)**

1. Wash the cells with PBS at room temperature.
2. Add Accutase™ cell detachment solution to adequately cover the entire area of the culture vessel.
3. Incubate at room temperature for 5 to 10 minutes, or until cells are detached. Cells can also be incubated at 37°C, however enzyme activity will decrease over time.
    a. Additional time and/or Accutase™ may be needed to disassociate three-dimensional structures.
4. Triturate the cells to aid in obtaining a single-cell suspension.
    a. Additional cell culture medium may be added to assist in obtaining a single-cell suspension.
    b. The additional cell culture medium will neutralize the Accutase™ and allow for passaging of cells without additional washes.
5. Remove a small subset of the cell suspension and examine under a microscope to confirm the presence of single cells.

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Suggested Companion Products

Catalog Number	Name	Size	Clone
354277	BD Matrigel™ hESC-qualified Matrix	5.0 ml	(none)

Product Notices

1. Accutase is a registered trademark of Innovative Cell Technologies, Inc.
2. mTESR™1 is a trademark of StemCell Technologies.
3. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.

References

Bajpai R, Lesperance J, Kim M, Terskikh AV. Efficient propagation of single cells Accutase-dissociated human embryonic stem cells. *Mol Reprod Dev.* 2008; 75(5):818-827. (Methodology)

Emre N, Vidal JG, Elia J, et al. The ROCK inhibitor Y-27632 improves recovery of human embryonic stem cells after fluorescence-activated cell sorting with multiple cell surface markers. *PLoS ONE.* 2010; 5(8):e12148. (Methodology: Cell culture)

Lamerato-Kozicki AR, Helm KM, Jubala CM, et al. Canine hemangiosarcoma originates from hematopoietic precursors with potential for endothelial differentiation. *Exp Hematol.* 2006; 34(7):870-878. (Methodology: Cell culture)

Wachs FP, Couillard-Despres S, Engelhardt M, et al. High efficacy of clonal growth and expansion of adult neural stem cells. *Lab Invest.* 2003; 83(7):949-962. (Methodology: Cell culture)

Weikert C, Eppenberger-Eberhardt M, Eppenberger HM. Cellular engineering of ventricular adult rat cardiomyocytes. *Cardiovasc Res.* 2003; 59(4):874-882. (Methodology: Cell culture)

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