Accutase®XL
Lyophilized Accutase

Cell Detachment Reagent

Cat. # SCR104

pack size: 500ml

Store at -20°C

FOR RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES
NOT FOR HUMAN OR ANIMAL CONSUMPTION

Certificate of Analysis

Background

Accutase is a cell detachment solution of proteolytic and collagenolytic enzymes that is useful for the routine detachment of cells from standard tissue culture plasticware and adhesion coated plasticware, including SmartPlastic™. Accutase is direct replacement for trypsin cell detachment solution and does not require enzyme inactivation with serum. Accutase does not contain mammalian or bacterial derived products.

AccutaseXL is a lyophilized form of Accutase with extended stability. AccutaseXL, once reconstituted, will make 500ml of complete 1X Accutase solution.

Accutase has been shown to be effective on a wide variety of cell types including: 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 and D54, HT1080 fibrosarcoma cells, and Sf9 insect cells.

Source

Accutase is derived from a non-mammalian (invertebrate) source.

Presentation

1X Accutase enzymes in Dulbecco's PBS (0.2 g/L KCl, 0.2 g/L KH2PO4, 8 g/L NaCl, and 1.15 g/L Na2HPO4) containing 0.5 mM EDTA4Na and 3 mg/L Phenol Red.

Quality Control Assay

pH: 6.8-7.8

Activity Assay: 500-20 units/ml (1 unit will release 1 µmole pNA from SUCR-pNA per minute at 37˚C)

Functional Assay: Cell detachment assay

Storage and Handling

Store lyophilized AccutaseXL at -20°C for up to 4 months from date of receipt.

Once reconstituted in water, liquid Accutase can be aliquoted and frozen at -20°C for 2 years or stored for 2 months at 4°C. It is recommended to thaw liquid Accutase at 4°C overnight or in a bath of cool water.

Reconstitution Instructions

1. Place 400 mls of room temp dH2O into a 500 ml container. Do not use warm or hot water. Accutase is temperature sensitive.
2. Remove cap of lyophilized Accutase vial and add 40 mls of cold dH20 to vial. Recap and vortex 1-2 minutes or invert and swirl until dissolved.
3. Pipet the entire contents of the Accutase vial into the container from step 1. Use a pipet to insure complete transfer of contents.
4. Rinse the vial twice with 25 ml of cold dH2O and add to container.
5. Bring volume of container up to 500 ml with cold dH2O.
6. Mix well.
7. Aseptically filter the entire 500 mls through a 0.22micron filter (EMD Millipore SCGPU05RE).

General Cell Detachment Protocol

1. Aspirate the media from culture dish .
2. Wash once with PBS and add 2ml of Accutase to culture dish.
3. Incubate for 2 to 5 minutes at RT until individual single cells start to round up.
4. Gently rinse to remove cells off of the plate's surface.

SPECIES LEGEND: **H** Human **Ca** Canine **M** Mouse **R** Rat **Rb** Rabbit **B** Bovine **P** Porcine **WR** Most Common Vertebrates

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5. Transfer cell suspension to 15mL conical tube. Gently pipette up and down until cells are in a single cell suspension.

6. Add 8 ml of media to rinse any remaining cells off of the dish's surface and transfer to the conical tube from Step 5.

7. Take a 20µl sample of the cell suspension to determine viable cell density.

8. Centrifuge conical tube containing the cell suspension at 200g for 4 minutes.

9. Aspirate supernatant, resuspend in fresh medium and plate on coated dish(s). Incubate at 37˚C in a humidified 5% CO2 incubator.

References

1. Efficient Propagation of Single Cells Accutase-Dissociated human Embryonic Stem Cells, Bajpai, et al, Journal of Molecular Reproduction and Development, 2007, DOI 10.1002/mrd:1-10.

2. Human Embryonic Stem Cell-derived Dopaminergic Neurons Reverse Functional Deficit in Parkinsonian Rats, Yang, et al, Stem Cells, 2007; 0: 2007-0494v1.

3. Oxygen Reduces Accumulation of Type IV Collagen in Endothelial Cell Subcellular Matrix via Oxidative Stress, T. Brevig, et al, Artificial Organs, Volume 30 Issue 12 Page 915-921, December 2006.

4. Canine hemangiosarcoma originates from hematopoietic precursors with potential for endothelial differentiation, Lamerota-Kozicki et al., Experimental Hematology, Vol. 34 Pages 870-878, April 2006.

5. The JAK3 inhibitor WHI-P154 prevents PDGF-evoked process outgrowth in human neural precursor cells, Richards et al., Journal of Neurochemistry, Vol. 97 Page 201, April 2006.

6. Nuclear factor-ÊB controls the reaggregation of 3D neurosphere cultures in vitro, Widera et al., European Cells and Materials, Vol. 11, Pages 76-85, 2006.

7. Rescue Purification Maximizes the Use of Human Islet Preparations for Transplantation, Ichii et al., American Journal of Transplantation, Vol. 5, Pages 21-30, 2005.

Representative Lot Data

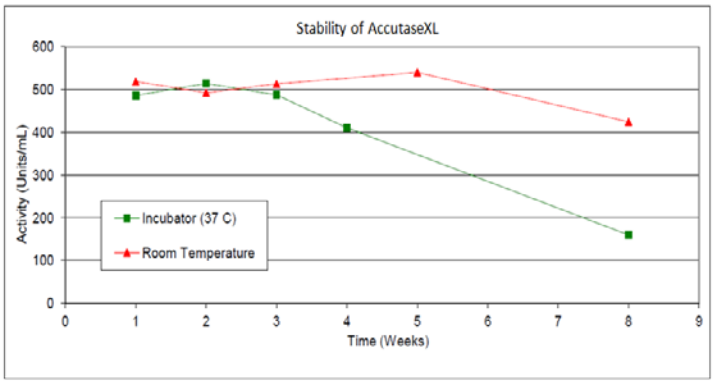

Figure 1. AccutaseXL stability testing using a standard enzyme activity assay. AccutaseXL stored at both room temperature (RT) and 37°C for over 3 weeks does not affect enzymatic activity.

RELATED PRODUCTS

cat #	description
SCR005	Accutase
SCR006	Accumax
TMS-006-A	EmbryoMax® Ultra Pure Water, sterile H2O
BSS-1006-A	EmbryoMax® 1X Dulbecco's Phosphate Buffered Saline w/o Ca++ & Mg++
CC095	Laminin, mouse purified
A-004-C	Poly-L-Ornithine Solution (0.01%)
A-003-E	Poly-D-Lysine solution, 1.0 mg/mL
A-005-C	Poly-L-Lysine Solution (0.01%)
SCR136	Animal Free Collagenase, Type A, 50mg
SCR137	Animal Free Collagenase, Type B, 50mg
SCR138	Animal Free Collagenase, Type C, 50mg
SCR139	Animal Free Collagenase/Dispase Blend I, 50mg
SCR140	Animal Free Collagenase/Dispase Blend II, 50mg