ACCUTASE® GMP

innovative cell technologies

Accutase® GMP, Cell Detachment Solution, is formulated with the exact same ingredients as our original product, Accutase, which has been manufactured by ICT for last 20 years. Accutase GMP is a ready-to-use cell detachment solution of proteolytic and collagenolytic enzymes. It is manufactured in an ISO-13485 certified and GMP compliant facility to ensure end to end traceability and lot to lot consistency. Accutase GMP can be supplied with a harmonized documentation package including certificates of analysis, certificates of origin, BSE/TSE statement and authorization letters for our Drug Master File, as appropriate. Useful for the routine detachment of cells from standard tissue culture plastic ware and adhesion coated plastic ware, and polymers. Accutase GMP performs exceptionally well in detaching cells for the analysis of cell surface markers, virus growth assays, 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 GMP 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, MRC5, 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 GMP does not contain mammalian or bacterial derived components.

Intended Use
Accutase GMP is a direct replacement for trypsin cell detachment solution. For research or further manufacturing use only. CAUTION: Not intended for human or animal diagnostic or therapeutic uses.

Precautions
Do not store Accutase GMP at room temperature. Accutase GMP is stable when stored at 2 to 8C for 2 months. It is recommended to thaw Accutase GMP at 4°C overnight or in a bath of cool water. **Do not thaw at 37C.**

Storage & Shelf Life
Store at -20C frozen, once defrosted store at 2-8C.
12 month shelf life from date of manufacture.

Formulation: 1X ACCUTASE GMP enzymes in Dulbecco's PBS without Ca^{++} Mg^{++} (0.2 g/L KCl, 0.2 g/L KH_2PO_4, 8 g/L NaCl, and 1.15 g/L Na_2HPO_4) containing 0.5 mM EDTA•4Na and 7.97 µM Phenol Red.

Note: Washing out or neutralizing of Accutase GMP is not required in routine cell passaging.

General Dissociation:

1. Aspirate the media from cell culture flask and wash with 4mL of DPBS (w/o calcium and magnesium). Remove DPBS
2. Add enough Accutase GMP to flask to completely cover the bottom of the flask (10 ml per 75cm^2 surface area).
3. Allow cells to detach at room temperature (RT) 5 to 10 minutes, up to a maximum of 1 hr. Or cells can be left on ice for several hours.
4. Smack the flask against palm of hand.
5. Take a 20µl sample of the cell suspension to determine the viable cell density.
6. Resuspend in fresh media and split into new flasks. Incubate at 37C in a humidified 5% CO_2 incubator.

Dissociation of human ESCs grown in Serum Free Media on hESC-qualified Basement Membrane Extract

1. Aspirate the media from culture dish and wash with 4mL of DPBS (w/o calcium and magnesium).
2. Aspirate DPBS and add 2mL of Accutase GMP 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.
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 37C in a humidified 5% CO_2 incubator.

Dissociation of adherent human or rat neuronal stem cells grown in Serum Free Media on coated dishes

1. Aspirate the media from the culture dish and wash with 4 ml of DPBS (w/o calcium and magnesium)
2. Aspirate DPBS and add 2ml of Accutase GMP 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.
5. Transfer cell suspension to 15 ml conical tube. Gently pipette up and down until all 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 the viable cell density.
8. Centrifuge conical tube containing the cell suspension at 200g for 4 minutes.
9. Aspirate supernatant, resuspend in fresh media and plate on coated dish(s). Incubate at 37C in a humidified 5% CO_2 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.
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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.

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CERTIFICATE OF ANALYSIS

PRODUCT NAME: Accutase® GMP, Sterile, Cell Detachment Solution

PART NUMBER: AT-107-100 ml **LOT NUMBER**: 2V0901A

DATE OF MANUFACTURE: 09-AUG-2022 **DATE OF EXPIRY**: 08/2023

STORAGE TEMP: -20ºC until defrosting then 4ºC

FORMULATION BUFFER: 2.67 mM Potassium Chloride, 1.47 mM Potassium Phosphate monobasic, 137.93 mM Sodium Chloride, 8.1 mM Sodium Phosphate dibasic, pH 7.4, 0.5 mM EDTA, 7.97 µM Phenol Red

Test	SPECIFICATION	This Lot
Appearance	Particulate free, clear, light pink solution	Pass
pH	7.2 to 7.5	7.3
Trypsin Activity	500 - 720 EAU/min/ml Enzyme Activity Units	699
Chymotrypsin/Elastase Activity	500 - 900 EAU/min/ml Enzyme Activity Units	682
Collagenase Type 1 Activity	350 -750 RFU/min/ml Relative Fluorescence Units	490
Cell Detachment Activity	Positive Result	Pass
Endotoxins, USP <85>	≤ 0.5 EU/ml	≤ 0.03
Sterility Testing, USP <71> (Membrane Filtration)	No evidence of microbial growth detected	Pass

Accutase GMP is manufactured in a facility which adheres to Good Manufacturing Practices under our **ISO13485:2016** certified Quality Management System. Get a copy of the ISO Certificate.
http://www.accutase.com/iso-134852016-

Kevin Barber	19-OCT-2022
Director, Quality Assurance	Date

Questions? info@innovativecelltech.com

For research applications or in the manufacturing of Cell, Gene or Tissue Based Products. No Warranty of this product is made, expressed, or implied including but not limited to the warranties of merchantability and fitness for a particular purpose.

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