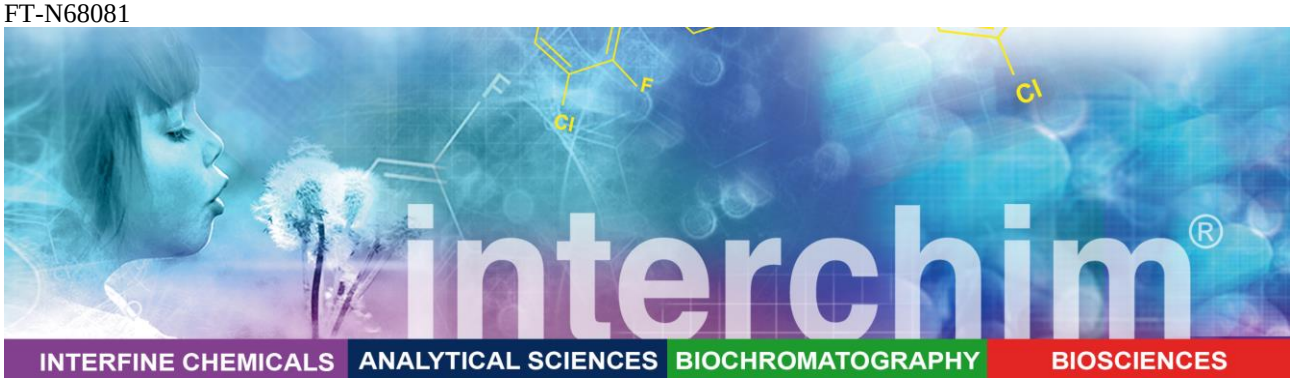
FT-N68081



Accutase, Cell Detachment Solution

Product Information

Catalog number: UPN68081, 100ml

Name: Accutase, Cell Detachment Solution
a cell detachment solution combining proteolytic and collagenasic enzymes
Formulation: 1X ACCUTASE enzymes in Dulbecco's PBS (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 3 mg/L Phenol Red.

Storage: Store at –20°C (shelf life up two years), shipped with blue pack (J)
After thawing, can be stored for up 2 months at +4°C
DO NOT STORE AT ROOM TEMPERATURE

Quality Control: Each lot is controled for sterility, and for functional activity of cell detachment.

Benefits:
(*over Trypsin method)
Detaches adherent cells in minutes
Dissociates cell gently* and rapidly
Ready-to-use*
No wash or neutralization necessary*
Lower risk of adventitious agents: not mammalian or bacterial derived products*
Maximum cell viability and plating efficiency, with improved cell morphology*
Protection of surface markers (epitopes, receptors,...)

Applications - Techniques:

Accutase is useful for the routine **detachment of adhering cells from cultures**, standard tissue culture plasticware and adhesion coated plasticware, including SmartPlastic™. It's a great alternative to trypsin. Accutase is particularly useful for detaching cells grown in cell culture media that does not contain serum, including Serum Free Media and Protein Free Media.Accutase can also dissociate cells from tissues.

Accutase performs exceptionally well in detaching cells for **functional assays**: analysis of cell surface markers, virus growth assay, cell proliferation, quiescence assays by serum starvation, transformation assays by oncogene transfection, neural crest cell migration assays, tumor cell migration assays, cell-cell recognition assays, cell haptotaxsis, routine cell passage, production scale-up (bioreactor), toxicological tests and flow cytometry analysis.

Applications - Cell Lines:

Accutase has been shown effective on : 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, Hu embryonic kidney 293 cells, L929 cells, immortalized mouse testicular germ

cells, 3T3, Vero, COS, HeLa, NT2, MG63 Hu osteosarcoma, M24 and A375 metastatic melanoma, gliomas U251 and D54 Hu gliomas, HT1080 fibrosarcoma, and Sf9 insect cells.

Directions for Use

Replacing Trypsin/EDTA: Accutase can replace Trypsin/EDTA for the detachment and dissociation of cells. The volumes used and methods employed will not need to be modified when replacing Trypsin/EDTA with Accutase.
The advantages Accutase offers over traditional trypsinisation include
1. Ease of use.
2. auto-inhibits at 37°C without the need for a neutralizing solution like with trypsin
3. Diminished cell damage and improved cell yield.
4. Enhanced cell viability.
5. Enhanced plating efficiency.
6. Improved cell morphology (qualitative).
7. Improved cell growth characteristics.

-Thaw Accutase at room temperature (or +4°C, or 37°C)
-Wash plate, flask or beads with sterile PBS
-Add Accutase to culture dish or flask using aseptic procedures at 3-10ml per 75cm^2 surface area
-Incubate at room temperature to detach 5-10minutes (or for faster detachment return culture to 37°C incubator)
-count cells and passage as usual: no additional washes or enzyme inhibitor are required in most cases.

Tissue Disaggregation: Tissue disaggregation can be performed in Accutase. Tissue disaggregation is best carried out between 4-25°C. The incubation time should be optimised depending on the tissue and culture time. As a guide incubation at 4°C should be conducted overnight (12-16 hrs) and those at 25°C for 1-2 hrs.
Alternatively, try Accumax (see below).

Functional assays: Use Accutase in the same manner that trypsin EDTA solutions are used to detach adherent cells from surfaces. Always handle cells with standard aseptic techniques if the cells are subcultured or propagated further.

Additional information

Accutase is a cell detachment solution which has been developed to meet the most demanding requirements for gentle and effective detachment of adherent cells. The combination of protease and collagenolytic activities maximize its versatility for cell detachment and tissue dissociation. No scrapping is required, no clumping is observed. 100% of cells are recovered after 10minutes, without harm in leaving cells for up to 45min, thanks to autodigestion of Accutase.

Due to its non-mammalian source (enzymes are from an invertebrate species), Accutase is guaranteed free of BSE, Parvovirus and other viruses common in trypsin. It is formulated in Dubelcco's PBS (0.2g/L KCl, 0.2g/L KH_2PO_4 ; 8g/L NaCl, 1.15g/L Na_2HPO_4), 0.5mM EDTA.$_4$Na, 3mg/L PhenolRed, and provided sterile filtered, ready to use.

Efficiency has been proven in detaching a variety of cell types, including primary fibroblasts, endothelial cells, neurons, tumour cell lines and insect cells. However, Accutase may not lift off all cell lines, as trypsin will not. Most cell lines when they are at a low passage number will lift off immediately with Accutase, but certain cell lines at a high passage number are difficult to lift off and more Accutase is required. This is likely to be caused by the formation of inter-cellular functional contacts between cells and contacts between cells and the culture flask. This formation is time and cell line dependent. In general: the longer you culture, the more junctions are formed, the more effort it takes to detach the cells.

In case of insufficient efficacy, we would recommend to try Accumax #UPN68091 for cell clumps detachment and tissue dissociation, which has a higher concentration of enzymes in it. Additionally, you may add enough EDTA to the Accutase or Accumax (which contains none) to give a 0.5mM concentration. The efficacy can be potentiated by up to a factor of 3.

Because Accutase gently detaches adhering cells and is not dose or time critical, cells remain viable for further propagation. Accutase is useful for enzymatic separation of viable tissue fragments from early chick embryo.

Cell membranes and surface active biomolecules (epitopes, receptors,...) will not be harmed and the structural and functional quality of the surface proteins remain intact, at the opposite of what happens frequently with Trypsin method.

Cell viability can be assessed advantageously using UptiBlue #UP669412 reagent

Literature (more on inquire):

Monferran S. *et al*., The membrane form of the DNA repair protein Ku interacts at the cell surface with metalloproteinase 9, *EMBO J*., **23**, 3758 (2004)

Pacciarini F. *et al*., Persistent Replication of Severe Acute Respiratory Syndrome Coronavirus in Human Tubular Kidney Cells Selects for Adaptive Mutations in the Membrane Protein, *J. Virol*., 82: 5137 - 5144 (2008) Abstract

Related products and documents:

Accutase is available in a lyophilized form (#WU8871-500mg) and GMF grade.

Legals:

See BioSciences Innovations catalogue and e-search tool.
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