Accutase®

Accutase®, Cell Detachment Solution, is a ready-to-use cell detachment solution of proteolytic and collagenolytic enzymes. Useful for the routine detachment of cells from standard tissue culture plastic ware and adhesion coated plastic ware, and polymers. Accutase performs exceptionally well in detaching cells for the analysis of cell surface markers, virus growth assay, 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 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 does not contain mammalian or bacterial derived products.

Intended Use

Accutase is direct replacement for trypsin cell detachment solution. For research use only.

CAUTION: Not intended for human or animal diagnostic or therapeutic uses.

Precautions

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

Storage & Shelf Life

Store at -20℃ frozen. 2-8℃ defrosted. 24 month shelf life frozen.

Formulation: 1 x 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.

Use:

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

General Dissociation:

1. Aspirate the media and wash with 4 mL of DPBS (w/o calcium and magnesium).
2. Add Accutase to flask (10 mL per 75cm^2 surface area) using aseptic procedures.
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 20 µL sample of the cell suspension to determine the viable cell density.

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6. Resuspend in fresh media and split into new flasks. Incubate at 37℃ 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 2 mL 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.
5. Transfer cell suspension to 15 mL 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 20 µL 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℃ 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 2 mL of Accutase to culture dish.
3. Incubate for 2 to 5 minutes at RT until individual single cell 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 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 20 µL 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 37℃ in a humidified 5% CO_2 incubator.

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