

**Accutase®**

#GTC01.0100 (100ml)

(FOR RESEARCH ONLY)



**Product:** Accutase® is a ready-to-use non-mammalian, non-bacterial replacement for all applications of trypsin. Accutase® is a natural enzyme mixture with proteolytic and collagenolytic enzyme activity. This means it mimics the action of trypsin and collagenase at the same time. However, because it is more efficient than mammalian trypsin & collagenase, it is formulated at a much lower concentration making it less toxic and gentler, but just as effective.

Can be used whenever gentle and efficient detachment of any adherent cell line is needed. Accutase® is a direct replacement for trypsin. Works extremely well on embryonic and neuronal stem cells; mono layers of stem cells can be grown after passaging with Accutase®. Preserves most epitopes for subsequent flow cytometry analysis. Does not need to be neutralized when passaging adherent cells. The addition of more media after the cells are split dilutes Accutase® so it is no longer able to detach cells. Does not need to be aliquoted. A bottle is stable in the refrigerator for 2 months

Accutase® performs exceptionally well in detaching cells for: hESC culturing, 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, apoptosis, cell haptotaxis, tumor cell migration assays, routine cell passage, production scale-up (bioreactor), and flow cytometry.

**Quantity:** #GTC01.0100 comprises 100ml of Accutase®.

**Applications:** ready-to-use cell detachment solution for breaking down cell adhesion structures located on the outside of cells that attaches them to plasticware.

**Appearance:** Accutase® contains phenol red as a pH indicator. If frozen, it should be yellow and upon thawing, it becomes an orange liquid.

**Storage:** -20°C. Once defrosted, store at +2°C-8°C for up to 2 months. If needed, one can freeze the solution again, promptly after using. Accutase® should be defrosted overnight in a refrigerator or placed in a cold-water bath. Accutase® should NOT be defrosted in a warm water bath as the enzyme mixture is sensitive to temperatures above 37°C and enzymes will be inactivated at 37°C after 45 minutes. After defrosting, mix the solution well, as the components will not melt evenly (one can notice a colour gradient), and use promptly. Store the remaining solution in the refrigerator as indicated.

*Accutase® is a registered trademark of Innovative Cell Technologies, Inc.*

**Prior to use:**

Thaw Accutase® as indicated above. It should be used cold and not pre-heated to 37°C. The entire protocol should be carried out in a laminar flow hood, using proper aseptic techniques.

**Typical Cell Passaging Protocol**

1. Carefully aspirate off all the culture media from the flask. There is no need to rinse with PBS.
2. Immediately after, add sufficient Accutase® to cover the cells (2-5ml for a T25 flask), and set the flask aside at room temperature for 5-10 minutes (max 1 hour). Regularly, check cells visually to verify if cell have rounded instead of shrunken and no longer appear “spidery” whilst remaining attached to the flask.
3. Once cells are detached, beat the flask against the palm of your hand to loosen any remaining attached cells
4. Gently disperse the cells. Take a sample to determine the viable cell density, and add aliquots of detached cells to fresh culture media in new flasks. There is no need for neutralization. Depending on the cell line, cells will reattach within a few minutes.

**For more specific protocols, please visit our website**