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## Technical Info

# ACCUTASE™

### For cell dissociation - a gentle alternative to trypsin

The detachment of cells from culture surfaces for further cultivation or cell analysis is an unavoidable step in cell culture. Conventional dissociation reagents such as trypsin or trypsin/EDTA solutions are widely used as common tools in routine cell culture. Nevertheless, trypsin solutions attack the cell surface and affect cell viability. In order to minimize cell damage as much as possible, an inhibition step of this enzyme must follow trypsinization.

Cell dissociation in general is a stressful process for cells. Even though most cells recover, cell stress should be kept as low as possible. Alternatives to trypsin, such as Accutase solution, are already available for this purpose.

Accutase™ is a natural mixture of proteolytic and collagenolytic enzymes from crustaceans. The mechanism of action is thus comparable to that of trypsin and collagenase simultaneously and can thus be used as a direct substitute for both enzymes. Accordingly, the action of Accutase™ takes place not only on the cell, but also on the proteins and collagens of the extracellular matrix. In addition to cell detachment from culture surfaces, Accutase™ can also be used for the isolation of cells from animal tissue.

### Why is Accutase™ the gentler choice for cell dissociation?

Due to its much higher efficiency compared to trypsin and collagenase, Accutase™ can be used at a much lower concentration. The cell surface is thus attacked significantly less, so that the membrane proteins remain intact. This not only achieves high cell yield, but also high cell viability for more efficient follow-up analyses such as FACS. A subsequent inhibition step by trypsin inhibitors or serum is not necessary. By adding fresh culture medium, the Accutase™ concentration is already low enough that no further effect takes place.

Accutase™ has also been shown to act very efficiently on sensitive cells, such as embryonic and neural stem cells.

The ROTI®Cell Accutase solution is a ready-to-use solution, which allows direct usage. Compared to trypsin solutions, it is stable for two months at +4°C, so no aliquoting is required.

### The advantages at a glance:

- Direct replacement for trypsin applications
- Gentle detachment of cells, also suitable for sensitive cells
- Reduced cell stress with maximum recovery and yield
- Membrane proteins remain intact for subsequent analyses
- No neutralization step by inhibitors necessary
- Without mammalian components
- Stable at +4°C for two months

### Tested cell lines:

Fibroblasts, keratinocytes, vascular endothelial cells, hepatocytes, vascular smooth muscle cells, hepatocyte progenitor cells, primary neuronal cells from chicken embryos, bone marrow stem cells, human embryonic 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 cells, U251 and D54 gliomas, HT 1080 fibrosarcoma cells and Sf9 insect cells.



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### Recommended protocol:

The ROTI®Cell Accutase solution can be used directly in place of trypsin solutions in any cell dissociation protocols. A detailed example protocol for cell passage / subcultivation of cells can be found on the product pages of our trypsin solutions under downloads.

Differences in the use of Accutase™ compared to trypsin:

1. Never thaw a bottle of Accutase™ at 37 °C. A thawed bottle of Accutase™ can be removed from the refrigerator and immediately applied to the cells.
2. Volume and incubation times: incubate 2.5 to 5 ml on 25 cm<sup>2</sup> of cell lawn (depending on confluence and density of cells) at room temperature for 5 to 10 minutes up to a maximum of 1 hour.
3. Centrifugation and inhibition step not required. Cell suspension can be added directly to new cell culture flasks with fresh medium.

### Composition of the ROTI®Cell Accutase solution:

Ingredients	Concentration mg/L
EDTA·4Na	220
KCl	200
KH <sub>2</sub> PO <sub>4</sub>	200
NaCl	8000
Na <sub>2</sub> HPO <sub>4</sub>	1150
Phenol Red	3
Accutase	1x conc.

LH, 24.01.23