

To recover cells from tissues, clumps and from culture recipients before culturing or analysis, several methods have been proposed. Cells should be ideally preserved. Enzymatic proteolysis (by trypsin i.e.) has become a standard technique. Interchim offers conventional enzymes, as well a very mild and efficient enzymatic reagent, Accutase, ideal for cell detachment for culture plates, and Accumax, designed for clumps dissociation before FCM analysis.

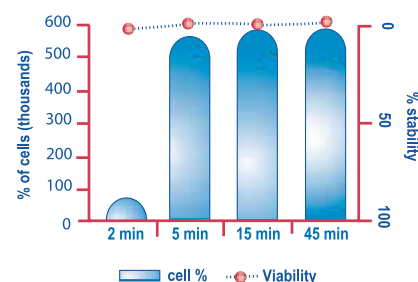
Accutase, cell detachment solution

- ◆ Detaches Adherent Cells in Minutes
- ◆ Dissociates Tissues for Primary Cell Culture
- ◆ Gentle Cell Detachment for Maximum Cell Viability
- ◆ Highest Plating Efficiency
- ◆ Cost Effective and Ready-to-Use

Accutase has been developed to meet the most demanding needs for gentle and effective detachment of adherent cells, ad fibroblasts, endothelial cells, neurons, tumor cell lines, and insect cells.

Accutase gently and rapidly dissociates tissues for cell isolation and propagation. It is recommended for use with SmartPlastic adhesion cell culture plasticware. Accutase combines protease and collagenolytic activities which maximizes its versatility for cell detachment and tissue dissociation. It does not contain mammalian or bacterial-derived products.

| Description | Cat.# | Qty |
|-------------|----------|--------|
| Accutase | UPN68081 | 100 ml |



CELL DETACHMENT : Human MG63 fibrosarcoma cells cultured on tissue culture treated dishes in DMEM + 10% FBS were treated with ACCUTASE. Treatment results in rapid cell detachment, a single cell suspension, and high viability. ACCUTASE is gentle on cells ; viability was $97 \pm 3\%$ even after 45 minutes in ACCUTASE.



Proteolytic Enzymes

Trypsin (Porcine pancreas)

- ◆ Special preparation for single cell suspension
- ◆ Tissue culture grade

Trypsin is a pancreatic serine protease with substrate specificity based upon positively charged lysine and arginine side chains. It is derived from a 34 kDa inactive precursor zymogen, trypsinogen, after enzymatic removal of an n-terminal 6-amino acid leader sequence resulting in the 23.8 kDa trypsin molecule. The optimum pH is 8.0. Trypsin is inhibited by organophosphorus compounds such as diisopropylfluorophosphate and natural inhibitors from pancreas, soybean, lima bean, and egg white. Trypsin cleaves amide and ester bonds of Arginine and Lysine.

| Description | Cat.# | Qty |
|--------------------------|--------|-----------------------|
| Trypsin 1:250 | N12610 | 25 g |
| | N12611 | 50 g |
| | N12612 | 250 g |
| Trypsin 1:300 | N12300 | 25 g |
| | N12301 | 50 g |
| | N12302 | 250 g |
| Trypsin proteomics grade | 243872 | 1 g |
| | 243877 | 5 g |
| Trypsin proteomics grade | 243872 | 1 g |
| | N15151 | 10 g |
| | N15152 | 10 g proteomics grade |
| | N15153 | 1 g proteomics grade |

Technical tip

Enzyme that acts to degrade proteins is referred to as a proteolytic enzyme, or proteinase. Trypsin is one of the three principal digestive proteinases, the other two being pepsin and chymotrypsin. In the digestive process, trypsin acts with the other proteinases to break down dietary protein molecules to their component peptides and amino acids. Trypsin continues the process of digestion (begun in the stomach) in the small intestine where a slightly alkaline environment (about pH 8) promotes its maximal enzymatic activity. Trypsin, produced in an inactive form by the pancreas, is remarkably similar in chemical composition and in structure to the other chief pancreatic proteinase, chymotrypsin. Both enzymes also appear to have similar mechanisms of action; residues of histidine and serine are found in the active sites of both. The chief difference between the two molecules seems to be in their specificity : each is active only against the peptide bonds in protein molecules that have carboxyl groups donated by certain amino acids. For trypsin these amino acids are arginine and lysine, for chymotrypsin they are tyrosine, phenylalanine, tryptophan, methionine, and leucine. Trypsin is the most discriminating of all the proteolytic enzymes in terms of the restricted number of chemical bonds that it will attack. Chemists have made good use of this fact to determine the amino acid sequence of proteins, as well cell biologists to dissociate cells.

Cell Biology - Culture

Cell Recovery

Chymotrypsin

Chymotrypsin is a proteolytic, or protein-digesting, enzyme active in the mammalian intestinal tract. Peptides are further split into free amino acids. Chymotrypsin is produced in the pancreas as the inactive, or zymogen, form chymotrypsinogen. Along with other digestive enzymes of the pancreas, chymotrypsinogen is carried in the pancreatic juice through the pancreatic duct into the duodenum. There chymotrypsinogen is activated by another enzyme, trypsin, and by molecules of active chymotrypsin. Partly because it was one of the first enzymes available commercially in crystalline form, chymotrypsin has been studied extensively.

Chymotrypsin preferentially catalyzes the hydrolysis of peptide bonds involving L-isomers of tyrosine, phenylalanine and tryptophan. It also readily acts upon amides and esters of susceptible amino acids. Chymotrypsin catalyzes the hydrolysis of bonds of leucyl, methionyl, asparaginyl and glutamyl residues

| Description | Cat.# | Qty |
|--------------------------------|--------|----------------------|
| Chymotrypsin | N12160 | 1 g |
| | N12161 | 5 g |
| Chymotrypsin, proteomics grade | 571762 | 1 g proteomics grade |
| | 571763 | 5 g proteomics grade |

Pepsin

Pepsin is an enzyme produced in the mucosal lining of the stomach that acts to degrade protein. In the laboratory studies pepsin is most efficient in cleaving bonds involving the aromatic amino acids, phenylalanine, tryptophan, and tyrosine. Pepsin is synthesized in an inactive form by the stomach lining; hydrochloric acid, also produced by the gastric mucosa, is necessary to convert the inactive enzyme and to maintain the optimum acidity (pH 1-3) for pepsin function. Pepsin is an acidic protease. Its inactive zymogen precursor, pepsinogen, is produced in the stomach mucosa. There are several pepsins designated A, B, C, and D. Pepsin A, the major component, has a molecular weight of 35 000 daltons and an optimum pH of approximately 1.0 for substrates such as casein or hemoglobin if the substrate is native protein.

| Description | Cat.# | Qty |
|---------------------------------|--------|-------|
| Pepsin 1:3000 | 398890 | 250 g |
| | 398891 | 500 g |
| Pepsin 1:3000, proteomics grade | FM2178 | 250 g |
| | FM2179 | 500 g |

Accumax, for cell disaggregation & cell counting

- ◆ Dissociates Clumped Cells in Minutes
- ◆ Results in Single Cell Suspension
- ◆ Gentle Cell Disaggregation for Maximum Cell Viability
- ◆ Yields Accurate, Reproducible Cell Counts
- ◆ Cost Effective Available Ready-to-Use

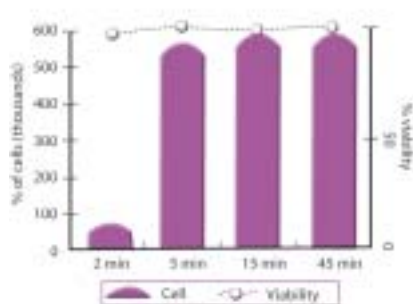
Accumax, developed to meet the most demanding needs for an effective cell aggregates dissociation solution. It has been proven effective in dissociating clumped cells in suspension cultures of hybridomas, CHO, BHK, 293, and others.

Accumax gently and rapidly dissociates cell clumps to yield single cell suspensions for accurate and reproducible cell counts.

Accumax combines protease, collagenolytic, and DNase activities, which maximizes its versatility for cell aggregates dissociation.

Accumax does not contain mammalian or bacterial-derived products. It is available as a ready-to-use liquid, quality controlled for in vitro cell culture applications.

| Description | Cat.# | Qty |
|-------------|----------|--------|
| Accumax | UPN68091 | 100 ml |



Various constructs of genetically engineered CHO cells, BHK cells, and a hybridoma were grown in suspension in serum-free or protein-free medium. Representative cells aliquots were treated with an equal volume of PBS or ACCUMAX and incubated for 5 minutes at 37°C. Cell number was then determined with a Coulter Counter.

