



MP Biomedicals, LLC

29525 Fountain Parkway  
Solon, Ohio 44139

Telephone: 440/337-1200  
Toll Free: 800/854-0530  
Fax: 440/337-1180  
mailto: [biotech@mpbio.com](mailto:biotech@mpbio.com)  
web: <http://www.mpbio.com>

## TECHNICAL INFORMATION

Catalog Number: 1689149, 1689649, 1689154, 1689654

### Trypsin-EDTA Solutions

**Molecular Weight:** Approximately 23,000 (trypsin)

**CAS #** 9002-07-7 (trypsin)

**EC #** 3.4.21.4 (trypsin)

**Source:** Porcine (for trypsin)

**Activity:**

One BAEE unit will produce a DA<sub>253</sub> of 0.001 per minute at pH 7.6 at 25°C using BAEE as substrate. Reaction volume = 3.2 ml (1 cm light path).

One TAME unit hydrolyzes 1 umole of p-toluene-sulfonyl-L-arginine methyl ester (TAME) per minute at 25°C, pH 8.2, in the presence of 0.001 M calcium ion.

One USP trypsin unit is the activity causing a change in absorbance of 0.003 per minute under the conditions specified.

**Activity Conversion:**

1 TAME unit = 19.2 USP or NF units = 57.5 BAEE Units

**Composition:** Trypsin is composed of two subunits, a-trypsin and b-trypsin. a-Trypsin is composed of two peptide chains and b-trypsin is composed of one chain.

**Inhibitors:** Trypsin is inhibited by organophosphorus compounds such as diisopropyl fluorophosphate and natural trypsin inhibitors from pancreas, soybean, lima bean and egg white. Silver ions are also potent inhibitors. Specific inhibitors are AEBSF, antipain, aprotinin, DFP, leupeptin, PMSF, TLCK, and Trypsin Inhibitor.

**Specificity:** The protease activity of trypsin is highly specific toward positively charged side chains with lysine and arginine.<sup>11,16</sup> Forms complexes with a<sub>2</sub>-macroglobulin.<sup>9</sup> Can be used in the isolation of intact, detergent-free phycobilisomes<sup>10</sup> and in the hydrolysis/condensation of carboxylic ester bonds.<sup>15</sup>

**Formulation (for #16891 - Trypsin 1:250 (0.05%) and 0.02% EDTA):**

Components	mg/L	Mol. Wt.	Mol. (mM)
<b>Inorganic Salts</b>			
EDTA 2Na 2H <sub>2</sub> O	200.00000	372.2	0.54
Potassium Chloride [KCl]	400.00000	74.55	5.37
Sodium Bicarbonate [NaHCO <sub>3</sub> ]	580.00000	84.01	6.90
Sodium Chloride [NaCl]	8000.00000	58.44	136.89
<b>Other</b>			
Dextrose	1000.00000	180.2	5.55
Phenol Red Sodium Salt	2.00000	376.4	0.01
Trypsin 1:250	500.00000	n/a	n/a

**Formulation (for #16896 - Trypsin 1:250 (0.25%) and 1 mM EDTA in Modified HBSS without Magnesium and Calcium):**

Component		Mol. Wt.	Mol. (mM)
<b>Inorganic Salts</b>			
<i>mg/liter</i>			
EDTA 2Na 2H <sub>2</sub> O		372.2	1.00
Potassium Chloride [KCl]	372.2000	74.55	5.37
	400.0000		

Potassium Phosphate Monobasic [KH <sub>2</sub> PO <sub>4</sub> ]		136.09	0.44
Sodium Bicarbonate [NaHCO <sub>3</sub> ]	60.0000	84.01	4.17
Sodium Chloride [NaCl]	350.0000	58.44	136.89
Sodium Phosphate Dibasic [Na <sub>2</sub> HPO <sub>4</sub> ]	8000.0000	141.96	0.33
<b>Other</b>	47.5000		
Dextrose		180.2	5.55
Phenol Red Sodium Salt	1000.0000	376.4	0.05
Trypsin 1:250	17.0000	n/a	n/a
	2500.0000		

#### Typical Protocol to Remove Adherent Cells from a Culture Surface:

- Remove the culture medium from the culture vessel by aspiration and wash the monolayer with a Ca<sup>2+</sup> and Mg<sup>2+</sup> free salt solution to remove all traces of serum. Remove the salt solution by aspiration.
- Dispense enough of the Trypsin-EDTA solution into the culture vessel to completely cover the monolayer of cells and place in a 37°C incubator for approximately 2 minutes.
- Remove the Trypsin-EDTA solution by aspiration and return the closed culture vessel to the incubator. The coated cells are allowed to incubate until the cells detach from the surface.

Progress can be checked by examination with an inverted microscope.

**NOTE:** The time required to remove the cells from the culture surface is dependent on the cell type, population density, serum concentration in the growth medium, potency of the trypsin and time since the last subculture. Trypsin causes cellular damage and time of exposure should be kept to a minimum.

- When the trypsinization process is complete, the cells will be in suspension and appear rounded.
- It is advisable to add serum or medium containing serum to the cell suspension as soon as possible to inhibit further tryptic activity which may damage the cells.
- Cells can be resuspended by gently pipetting the cell suspension to break up the cell clumps. Further dilution can be made, if required, for cell counts and/or subculturing.

#### Availability:

Catalog Number	Description	Size
1689649	1X Trypsin-EDTA 1:250, 0.25% (w/v)	100 ml
1689654	solution	500 ml
1689149	Trypsin-EDTA Solution	100 ml
1689154	0.05% (w/v) Trypsin (1:250) and 0.02% (w/v) EDTA	500 ml

#### Also Available:

Catalog Number	Description	Size
101179	Trypsin, source: beef pancreas; 2X crystallized, salt-free, lyophilized; approximately 3000 NF units or 180 TAME units/mg; Chymotrypsin approximately 3.5%	100 mg 500 mg 1 g 10 g
101192	Trypsin; source: beef pancreas; 3X crystallized; Sterile; approximately 3000 NF units/mg	50 mg
191340	Trypsin; source: human pancreas; activity approximately 1 unit/mg protein; supplied frozen in < 2 mM hydrochloric acid	25 ug 5 x 25 ug
150213	Trypsin, Immunohistology Grade; source: porcine pancreas; activity approximately 400 USP units/mg solid; Chymotrypsin activity approximately 95 USP units/mg solid. Can be used to enhance staining and to unmask antigens after routine fixation and processing.	1 g 10 g 100 g
190046	Trypsin; source: porcine pancreas; activity approximately 75000 to 125000 BAEE units/ml. This product is a 40X concentrate, sterile filtered and tested to assure that it is negative from microbial contaminants.	100 ml
103139	Trypsin, 1-250; source: porcine pancreas; lyophilized; activity approximately 250000 USP units/gm	25 g 100 g 250 g 500 g 1 kg

103140	Trypsin, 1-300; source: porcine pancreas; lyophilized; activity approximately 300000 USP units/gm	25 g 100 g 250 g 500 g 1 kg
101171	Trypsin, Acetylated; source: bovine pancreas; salt-free, lyophilized, 1X crystallized; approximately 2500 NF units or 150 TAME units/mg	25 mg 100 mg 250 mg 1 g
104922	Trypsin, DCC treated; source: bovine pancreas; Crystallized and treated with diphenylcarbamyl chloride to inhibit chymotrypsin	100 mg 250 mg 500 mg 1 g
1689349	Trypsin 1-250; 2.5% (w/v) solution in HBS, without calcium, magnesium and phenol red	100 ml
1689454	Trypsin 1-300; 0.25% solution in HBS with 200 IU/ml penicillin, 100 ug/ml streptomycin, and 0.5 g/L sodium bicarbonate, without calcium, magnesium and phenol red	500 ml

*Trypsin Related Products Available:*

Catalog Number	Description	Size
191324	Trypsin-Agarose	5 ml
100612	Trypsin Inhibitor from chicken egg whites	250 mg 500 mg 1 g 5 g
100798	Trypsin Inhibitor from lima beans	25 mg 100 mg 500 mg 1 g
101113	Trypsin Inhibitor from soybean	25 mg 100 mg 250 mg 500 mg 1 g 5 g
1676949	Non-Enzymatic Cell Dissociation Reagent	100 ml

**References:**

- Asgeirsson, B., Fox, J.W. and Bjarnason, J.B., "Purification and Characterization of trypsin from the poikilotherm *Gladus morhua*." *Eur. J. Biochem.*, **v. 180**, 85 (1989).
- Bergmeyer, H.U., Gawehn, K. and Grassl, M., in *Methods of Enzymatic Analysis*, Bergmeyer, H.U. (ed.), **v. 1, 2nd Ed.**, pp. 515-516, Academic Press, Inc., New York, NY (1974).
- Bode, W., *Naturwissenschaftler*, **v. 66**, 251 (1979).
- Cerovsky, V. and Jost, K., *Proc. Eur. Pept. Symp.*, **v. 17**, 395 (1983) (Oligomerization of the Ala-Ala-Arg tripeptide)
- Chambers, J., et al., "Silver ion inhibition of serine proteases. Crystallographic study of silver-trypsin." *Biochem. Biophys. Res. Comm.*, **v. 59**, 70 (1974).
- Cunningham, L., "Molecular-Kinetic properties of crystalline diisopropyl phosphoryl trypsin." *J. Biol. Chem.*, **v. 211**, 13 (1954).
- Feinmann, R.D., et al., *Ann. N.Y. Acad. Sci.*, **v. 421**, 178 (1983).
- Gebb, C., et al., *Dev. Biol. Stand.*, **v. 55**, 57 (1983). (Improved recovery in cell harvesting from microcarriers)
- Gonias, S.L. and Pizzo, S.V., *J. Biol. Chem.*, **v. 258**, 14682 (1983).
- Hiller, R.G., *Proc. 6th Int. Congr. Photosynth.*, **v. 3**, 351 (1984).
- Keil-Dlouha, V., et al., "Cleavage of Glucagon by a- and b-Trypsin." *FEBS Lett.*, **v. 16**, 287 (1971).
- Marquart, M., et al., *Acta Crystallogr.*, **Sect. B B39**, 480 (1983) (Geometry of the reactive site).
- Rick, W., *Method. Enzym. Anal.*, **3rd Ed.**, v. 1, 1052 (1974).
- Rothen, A., *Int. Rev. Cytol.*, **v. 80**, 267 (1982). (Interactions of solid-phase bound substrate with trypsin)
- Sakurai, T., et al., *J. Am. Chem. Soc.*, **v. 110**, 7236 (1988).
- Schwert, G.W. and Takenaka, Y., *Biochim. Biophys. Acta*, **v. 16**, 570 (1955).
- Vindeloev, L.L., et al., *Cytometry*, **v. 4**, 323 (1983). (Sample preparation for flow cytometric DNA analysis)
- Walker, J.E. and Keil, B., "Purification and characterization of different active forms of pork trypsin." *Eur. J. Biochem.*, **v. 32**, 486 (1973).
- *USP 24/NF 19*
- *Merck Index*, **12th Ed**, No9926
- Madin, S.H., Darby, N.B., Jr., *PSEBM*, **v. 98**, 574 (1958).
- Hanks, J.H., Wallace, R.E., *PSEBM*, **v. 71**, 196 (1949).