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ProductInformation

Trypsin-EDTA solution

Product Number **T 4049**
Storage Temperature -0 °C

Product Description
Enzyme Commission (EC) Number: 3.4.21.4
Molecular Weight: 23.4 kDa[1]
Extinction Coefficient: $E^{1\%}$ = 15.0 (280 nm)[2]
pI: 10.2 - 10.8[1,2]
Synonyms: Tryptase, Tryptar, Cocoonase, Parenzyme, Parenzymol

Trypsin Solution (2.5 g/l porcine trypsin and 0.2 g/l EDTA•4Na in Hank's Balanced Salt Solution with phenol red, 1X, cell cuture tested)

Trypsin consists of a single chain polypeptide of 223 amino acid residues. Trypsin is produced by the removal of the N-terminal hexapeptide from trypsinogen which is cleaved at the Lys^{6} - Ile^{7} peptide bond. The amino acid sequence of trypsin is cross-linked by 6 disulfide bridges. This native form of trypsin is referred to as β-trypsin. Autolysis of β-trypsin (which is cleaved at Lys^{131} - Ser^{132}) results in α-trypsin which is held together by disulfide bridges. Trypsin is a member of the serine protease family. The active site amino acid residues of trypsin include His^{46} and Ser^{183}.[1,3]

Trypsin will cleave peptides on the C-terminal side of lysine and arginine amino acid residues. The rate of hydrolysis is slower if an acidic residue is on either side of the cleavage site and no cleavage occurs if a proline residue is on the carboxyl side of the cleavage site. The pH optimum of trypsin is 7 - 9.[2] Trypsin will also hydrolyze ester and amide linkages of synthetic derivatives of amino acids such as: benzoyl L-arginine ethyl ester (BAEE), p-toluenesulfonyl-L-arginine methyl ester (TAME), tosyl-L-arginine methyl ester, Nα-benzoyl-L-arginine p-nitroanilide (BAPNA), L-lysyl-p-nitroanilide, and benzoyl-L-arginamide.[2,3,4,5] Assuming the pH and temperature are the same and using a molar extinction coefficient of 808 at 254 nm for BAEE, the following conversions are valid:

1 BAEE µM Unit = 200 BAEE Units
1 TAME µM Unit = 0.27 BAEE µM Units
1 BAEE µM Unit = 3.64 TAME Units
1 TAME µM Unit = 55 BAEE A_{253} Units
1 BAEE A_{253} Unit = 0.018 TAME µM Unit
1 TAME µM Unit = 180 TAME A_{247} Units
1 TAME A_{247} Unit = 0.33 BAEE Units
A USP Unit = ΔA_{253} of 0.003 per minute
1 NF Unit = 3.3 A_{253} BAEE Units.[9]

Note: These activity conversions were determined using bovine trypsin; however, they are thought to be similar for porcine trypsin.

Serine protease inhibitors that will inhibit trypsin include DFP (diisopropyl fluorophosphate), TLCK (Nα-p-tosyl-L-lysine chloromethyl ketone), PMSF (phenylmethanesulfonyl fluoride), APMSF(4-amidinophenylmethanesulfonyl fluoride), AEBSEF (4-(2-aminoethyl)benzenesulfonyl fluoride), aprotinin, leupeptin, α_2-macroglobulin, α_1-antitrypsin, p-aminobenzamidine, benzamidine (reversible), soybean trypsin inhibitor, lima bean inhibitor, bovine pancreas trypsin inhibitor, chicken egg white inhibitor, and turkey egg white inhibitor.[1,7]

Precautions and Disclaimer
For Laboratory Use Only. Not for drug, household or other uses.

Storage/Stability
This product is stored frozen.

This product does contain phenol red. The product is shipped on dry ice and there could be significant CO_2 buildup in the package. This CO_2 may enter the solution and lower the pH slightly, giving an orange (around pH 6.5) vs. pinkish (around pH 7.3) color. The solution, if orange (acidic) should still be good to use as is, or sodium hydroxide may be added to adjust the pH.

Trypsin retains most of its activity in 2.0 M urea, 2.0 M guanidine HCl, or 0.1% (w/v) SDS.[8] Trypsin is reversibly denatured at high pH (above 11), by precipitation with TCA, or by high concentrations of urea (greater than 6.5 M).[3] In order to abolish all trypsin activity, heating at 100 °C in 1% (w/v) SDS for 5 minutes is required.[9]

Procedure

Trypsin may be used to remove adherent cells from a culture surface. Cells are most commonly removed from the culture substrate by treatment with trypsin, or trypsin•EDTA. Trypsin 1X solutions can range from 0.025% to 0.5%. The reasons for the range of concentrations are as follows: (1) Differences in trypsin activity or potency; (2) Different incubation times; (3) Different cell lines.

Incubating cells with too high a trypsin concentration for too long a time period will damage cell membranes and kill the cells. If unsure about the concentration of trypsin to use, use a low concentration. There can be lot-to-lot variation in dissociation times which is to be expected since the enzymatic activity of each lot will differ. If trypsin is being solubilized or diluted from a concentrated solution, it is important to use a buffered salt solution that contains no Ca^{2+} or Mg^{2+}, such as Hank's Balanced Salt Solution, Modified (Product No. H 9394). Adjust the pH of trypsin solution to 7.4-7.6.

1) Remove medium from culture vessel by aspiration and wash the monolayer with Ca^{+2} and Mg^{+2}- free salt solution to remove all traces of serum. Remove salt solution by aspiration.
2) Dispense enough trypsin or trypsin•EDTA solution into culture vessel(s) to completely cover the monolayer of cells and place in 37 °C incubator for approximately 2 minutes.
3) Remove the trypsin or trypsin•EDTA solution by aspiration and return closed culture vessel(s) to incubator. The coated cells are allowed to incubate until cells detach from the surface. Progress can be checked by examination with an inverted microscope.
   Note: The time required to remove cells from the culture surface is dependent on cell type, population density, serum concentration in the growth medium, potency of trypsin and time since last subculture. Trypsin causes cellular damage and time of exposure should be kept to a minimum.
4) When trypsinization process is complete the cells will be in suspension and appear rounded.
5) 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 cells. Soybean trypsin inhibitor (Product No. T 9008) can also be added at an equimolar concentration to inhibit the trypsin that is present. Soybean trypsin inhibitor is used when culturing in serum-free conditions.
6) Cells can be resuspended by gently pipetting the cell suspension to break up the clumps. Further dilution can be made, if required, for cell counts and/or subculturing.

References

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