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TECHNICAL INFORMATION

Catalog Number: 101171, 101179, 101192, 103139, 103140, 104922, 150213, 153571, 1689349, 1689454, 190046, 191340

Trypsin

Molecular Weight: Approximately 23,000 or 22000 (for human)

CAS #: 9002-07-7

Physical Description: White to off white powder

EC # 3.4.21.4

Source: *Human, porcine* or *bovine pancreas*[6]

Activity:

One BAEE unit will produce a ΔA_{253} 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, α-trypsin and β-trypsin. α-Trypsin is composed of two peptide chains and β-trypsin is composed of one chain.

Optimum pH: Approximately 8.0

Extinction coefficient: $E^{1\%}_{280} = 14.3$

Isoelectric Point: pH 10.5[6]

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.[11,16] Forms complexes with α2-macroglobulin.[9] Can be used in the isolation of intact, detergent-free phycobilisomes[10] and in the hydrolysis/condensation of carboxylic ester bonds.[15]

Solutions: Solutions of trypsin should be thawed and swirled gently to mix. Solutions are relatively stable for periods of up to three months under refrigeration. If longer storage is desired, the concentrate should be aliquoted and refrozen. Thawed concentrate may contain a small amount of precipitate; this is normal and in no way will affect the efficacy of the product.

Solubility: Soluble in water; practically insoluble in alcohol and clycerol.

Formulation (for #16893 - Trypsin 1:250 in Hanks' Balanced Salt Solution without Magnesium and Calcium):

Components	*mg/L*	*Mol. Wt.*	*Mol. (mM)*
Inorganic Salts			
Potassium Chloride [KCl]	400.00000	74.55	5.37
Potassium Phosphate Monobasic [KH_2PO_4]	60.00000	136.09	0.44
Sodium Bicarbonate [$NaHCO_3$]	350.00000	84.01	4.17
Sodium Chloride [NaCl]	8000.00000	58.44	136.89
Sodium Phosphate Dibasic [Na_2HPO_4]	47.50000	141.96	0.33
Other			
Dextrose	1000.00000	180.2	5.55
Trypsin 1:250	25000.00000	n/a	n/a

Formulation (for #16894 - Trypsin 1:300, 0.25% Solution in HBS Solution (Modified) with 200 IU/ml Penicillin and 100 µg/ml Streptomycin, with 0.50 g/l Sodium Bicarbonate, without Calcium, Magnesium, and Phenol Red):

Ingredient	*mg/liter*	*Mol. Wt.*	*Mol. (mM)*
Inorganic Salts			
Potassium Chloride [KCl]	400.0000	74.55	5.37
Potassium Phosphate Monobasic [KH_2PO_4]	60.0000	136.09	0.44
Sodium Bicarbonate [$NaHCO_3$]	500.0000	84.01	5.95
Sodium Chloride [NaCl]	8000.0000	58.44	136.89
Sodium Phosphate Dibasic [Na_2HPO_4]	60.0000	141.96	0.42
Other			
Dextrose	1000.0000	180.2	5.55
Dihydrostreptomycin Sulfate	100.0000	730.7	0.14
Penicillin G Potassium Salt	200,000 units	372.5	n/a
Trypsin 1:300	2500.0000	n/a	n/a

Typical Procedures for the Removal of Adherent Cells from a Culture Surface:

Cells are most commonly removed from the culture substrate by treatment with trypsin, or trypsin-EDTA. If trypsin is being solubilized or diluted from a concentrated solution, it is important to use a buffered salt solution that contains no calcium or magnesium such as Hank's Balanced Salt Solution, Modified (MP catalog number 1810554 [1X HBS without calcium, magnesium and phenol red]). Adjust the pH of trypsin solution to 7.4 to 7.6.

EDTA is added to some trypsin solutions to intensify enzyme activity by chelating calcium and magnesium, thereby de-stabilizing the intercellular matrix.

Method 1:

1. Remove medium from culture vessel by aspiration and wash the monolayer with calcium and magnesium free salt solution to remove all traces of serum. Remove the salt solution by aspiration.

2. Dispense enough trypsin-EDTA solution into culture vessel(s) to completely cover the monolayer of cells.

   Trypsin-EDTA solution:

Balanced Salt Solution without calcium, magnesium or phenol red	500 ml
Trypsin	10 ml (typically a 2.5% w/v solution)
EDTA	5 ml of a 2% w/v solution

3. Pass the Trypsin/EDTA solution over the monolayer several times, by gently racking the flasks, and decant. Do not allow the Trypsin/EDTA solution to be in contact with the monolayer for longer than 30 seconds.

   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. Lay the flask flat and incubate at room temperature until the cells detach. Flasks which have been trypsinized should be inspected regularly to prevent the cells from remaining in the trypsin/EDTA for longer than is required.

5. Wash the cells off the base of the flask with 10 ml of the required growth medium, taking special care with cells which may still be adhering to the sides and shoulders.

6. Aspirate the cell suspension carefully to break down any cell aggregates. Avoid excessive frothing.

7. Proceed as per experimental protocol or split cells as required for stock.

8. At all times be aware of the possibility of cross contamination. NEVER mix caps or reuse a pipette.

Method 2:

Volumes given are for a 25 cm^2 T-Flask.

1. Decant supernatant fluid from the culture plate into a waste collection jar, taking care to use sterile technique.

2. Add 3 ml of cold trypsin (as per method 1, step 2) to the culture flask.

3. Incubate for 30 seconds (or longer, if necessary). Examine at low magnification. When it appears

that some of the cells have rounded up, but have yet to detach, decant the trypsin into a waste container. Continue to incubate the flask until virtually all cells have rounded up and can be readily dislodged.

4.
   a. Using a 10 ml pipette, add 10 mls fresh MEM (i.e. MP catalog number 12102) to the T-flask. Dispensing the MEM as a strong stream will aid in dislodging the cells.

      Note: 10 mls is sufficient for a 1:2 split.

   b. Using the same pipette, draw up the cell suspension and quickly dispense a 5 ml aliquot into two 25 cm^2 T-Flasks (one new, one old).

5. Incubate. Monitor the flasks periodically, beginning 30-45 minutes after inoculation. Rapid attachment (within about 1 hour) is indicative that the split has been successful.

6. Continue to monitor the culture's progress (i.e. "eyeball" the culture).

Method 3:

1. Drain and discard spent medium from the culture vessel (flask, petri dish, etc.).

2. Add a calcium and magnesium free balanced salt solution to the side of the culture vessel opposite the cells and gently swirl vessel to rinse cells. Aspirate the salt solution rinse to remove.

3. Add the trypsin solution to the side of the vessel opposite the cells (approximately 3 ml/25 ml flask) and gently swirl the vessel to cover the monolayer completely. Let cells sit for 1-2 minutes, and remove trypsin (NOTE: Before aspirating trypsin solution, make sure the monolayer is still intact.) Using trypsin at 4°C minimizes cell detachment at this phase.

4. Incubate cells for 1-2 minutes until the cells begin to round up. When the vessel is tilted, the monolayer should slide down the surface (timing may vary but it usually takes 5 to 15 minutes). Monitor the cells carefully. Forcing the cells to detach prematurely could result in clumping. Leaving trypsin too long will cause cell damage. A gentle tap may facilitate removal of more difficult to remove cell lines.

5. After detachment, drain cells to one side of the vessel. Add complete (i.e. containing serum) cell culture medium (approximately 0.1 to 0.2 ml per cm^2) or under serum free conditions, add a trypsin inhibitor, to neutralize the action of the trypsin. Disperse cells into suspension by pipetting repeatedly. The amount and intensity of pipetting will vary from one cell to another. Too vigorous pipetting may cause cell damage. If cells are too difficult to disperse without causing damage to cells, a stronger dissociating solution may be needed.

Typical Assay (BAEE activity): [2]

Principle:

$$\text{BAEE} + H_2O \xrightarrow{\text{Trypsin}} \text{N-}\alpha\text{-Benzoyl-L-Arginine} + \text{Ethanol}$$

where:

BAEE = N-α-Benzoyl-L-Arginine Ethyl Ester

Conditions:

T = 25°C
pH = 7.6
A253 nm
Light Path = 1 cm

Method: Continuous Spectrophotometric Rate Determination

Reagents:

A. 67 mM Sodium Phosphate Buffer, pH 7.6 at 25°C (Prepare 100 ml in deionized water using Sodium Phosphate Monobasic, Anhydrous, MP catalog number 195500. Adjust to pH 7.6 at 25°C with 1 M NaOH, MP catalog number 1688145).

B. 0.25 mM N-α-Benzoyl-L-Arginine Ester Solution (BAEE) (Prepare 50 ml in Reagent A using N-α -Benzoyl-L-Arginine Ethyl Ester Hydrochloride, MP catalog number 100088).

C. 1 mM Hydrochloric Acid Solution (HCl) (Prepare 50 ml in deionized using concentrated hydrochloric acid, MP catalog number 194054).

D. Trypsin Enzyme Solution (Immediately before use, prepare a solution containing 500 BAEE units/ml of Trypsin in cold Reagent C.)

Procedure:

Pipette the following reagents into suitable quartz cuvettes:

	Test	Blank
Reagent B (BAEE Solution)	3.00 ml	3.00 ml

Equilibrate to 25°C. Monitor the A_{253} nm until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent C (HCl)	0.00 ml	0.20 ml
Reagent D (Enzyme Solution)	0.20 ml	0.00 ml

Immediately mix by inversion and record the increase in $A_{253\ nm}$ for approximately 5 minutes. Obtain the $\Delta A_{253\ nm}$ /minute using the maximum linear rate for both the Test and Blank.

Final Assay Conditions:

In a 3.2 ml reaction mix, the final concentrations are 63 mM sodium phosphate, 0.23 mM BAEE, 0.06 mM hydrochloric acid and 100 units trypsin.

Calculations:

$$\text{BAEE units/ml enzyme} = \frac{(\Delta A_{253\ nm}/\text{min Test} - \Delta A_{253\ nm}/\text{min Blank})(df)}{(0.001)(0.20)}$$

where:

df = Dilution Factor
0.001 = The change in $A_{253\ nm}$/minute per unit of Trypsin at pH 7.6 at 25°C in a 3.2 ml reaction mix
0.20 = Volume (in mL) of enzyme used

$$\text{Units/mg solid} = \frac{\text{units/ml enzyme}}{\text{mg solid/ml enzyme}}$$

$$\text{Units/mg protein} = \frac{\text{units/ml enzyme}}{\text{mg protein/ml enzyme}}$$

Typical Assay (TAME unit):

Reagents:

A. 0.046 M Tris-HCl buffer, pH 8.1 with 0.0115 M calcium chloride.

B. 0.01 M TAME (N-α-p-Tosyl-L-arginine methyl ester hydrochloride, MP catalog number 103086)

C. 0.001 N HCl

D. Trypsin Enzyme Solution: Just before use dilute to a concentration of 10-20 ug/ml in Reagent C (HCl).

$$\text{mg of trypsin/ml} = A_{280} \times 0.70$$

Procedure:

Set spectrophotometer at 247 nm and 25°C.

Pipette into each cuvette as follows:

Reagent A (0.046 M Tris-HCl buffer, pH 8.1)	2.6 ml
Reagent B (TAME)	0.3 ml

Incubate in spectrophotometer at 25°C for 3-4 minutes to achieve temperature equilibration and establish a blank rate, if any. Add 0.1 ml diluted enzyme and record A_{247} for 3 to 4 minutes. Determine ΔA_{247} from initial linear portion of the curve. The reaction remains linear to an A_{247} of about 0.320. The reaction should be linear for at least three minutes. If this is not so repeat using less enzyme.

Calculation:

$$\text{U/mg protein} = \frac{\Delta A_{247}/\text{min X } 1000 \text{ X } 3}{*540 \text{ X mg Trypsin in reaction mixture}}$$

*540 equals extinction coefficient of N-α-p-Tosyl-L-arginine Methyl Ester at 247 nm.

Typical Assay (USP/NF):[19]

Reagents:

A. 0.067 M phosphate buffer, pH 7.6: Dissolve 4.54 g of monobasic potassium phosphate in water to make 500 ml of solution. Dissolve 4.73 g of anhydrous dibasic sodium phosphate in water to make 500 ml of solution. Mix 13 ml of the monobasic potassium phosphate solution with 87 ml of the anhydrous dibasic sodium phosphate solution.

B. Substrate Solution: Dissolve 85.7 mg of N-benzoyl-L-arginine ethyl ester hydrochloride (BAEE, MP catalog number 100088), suitable for use in assaying crystallized trypsin, in water to make 100 ml. Dilute 10 ml of this solution with 0.067 M phosphate buffer, pH 7.6 to 100 ml. Determine the absorbance of this solution, in a 1 cm cell, at 253 nm, in a suitable spectrophotometer equipped with thermospacers to maintain a temperature of 25 ± 0.1°C, using water as the blank. By the addition of 0.067 M phosphate buffer, pH 7.6, or of the Substrate solution before dilution, adjust the absorbance so that is measures not less than 0.575 and not more than 0.585. Use this substrate solution within 2 hours.

C. Crystallized Trypsin Solution: Dissolve a sufficient quantity of crystallized trypsin, accurately weighed, in 0.0010 N hydrochloric acid to obtain a solution containing about 50 to 60 USP trypsin units per ml.

Procedure:

Pipet 200 ul of 0.0010 N hydrochloric acid and 3.0 ml of the substrate solution into a 1 cm cell. Place this cell in a spectrophotometer, and adjust the instrument so that the absorbance reads 0.050 at 253 nm. Pipet 200 ul of crystallized trypsin solution, containing 10 to 12 USP trypsin units, into another 1 cm cell, add 3.0 ml of substrate solution, and place the cell in the spectrophotometer. At the time the substrate solution is added, start a stopwatch, and read the absorbance at 30 second intervals for 5 minutes. Repeat the procedure on the same dilution at least once. Plot a curve of absorbance against time, and use only those values that form a straight line to determine the activity of the crystallized trypsin. If the rate of change does not remain constant for at least 3 minutes, repeat the run, and if necessary, use a lower concentration. One USP trypsin unit is the activity causing a change in absorbance of 0.003 per minute under the conditions specified in this Assay. Calculate the number of USP trypsin units per mg taken by the formula:

$$(A_1 - A_2)/(0.003TW),$$

in which A_1 is the absorbance straight-line final reading, A_2 is the absorbance straight-line initial reading, T is the elapsed time, in minutes, between the initial and final readings, and W is the weight, in mg, of crystallized trypsin in the volume of solution used in determining the absorbances.

Availability:

Catalog Number	Description	Size
101179	Trypsin, source: beef pancreas; 2X crystallized, salt-free, lyophilized; approximately 3000 NF units or 180 TAME units/mg; Chymotrypsin	100 mg 500 mg

	approximately 3.5%	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 × 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, lyophilzed, 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
153571	Trypsin, 1-250; source: porcine pancreas; lyophilized; activity $\geq$ 250 units/mg, gamma irradiated to inactivate viral contaminants.	25 g 100 g 500 g 1 kg
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

Also Available:

Catalog Number	Description	Size
191324	Trypsin-Agarose	5 ml
1689649 1689654	1X Trypsin-EDTA 1:250 (0.25% w/v solution)	100 ml 500 ml
1689149 1689154	Trypsin-EDTA solution; 0.05% (w/v) trypsin 1:250 and 0.02% (w/v) EDTA	100 ml 500 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:

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