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Change to read:

▲▲ (Official 1-Dec-2022) Trypsin

▲(Title for this monograph—not to become official until December 1, 2022)

(Prior to December 1, 2022, the current practice of labeling the article of commerce with the name Crystallized Trypsin may be continued. Use of the name Trypsin will be permitted as of June 1, 2020; however, the use of this name will not be mandatory until December 1, 2022. The 30-month extension will provide the time needed by manufacturers and users to make necessary changes.)▲ (Official 1-Dec-2022)

Add the following:

▲

IVGGYTCGAN TVPYQVSLNS GYHFCGGSLI NSQWVVSAAH CYKSGIQVRL
GEDNINVVEG NEQFISASKS IVHPSYNSNT LNNDIMLIKL KSAASLNSRV
ASISLPTSCA SAGTQCLISG WGNTKSSGTS YPDVLKCLKA PILSDSSCKS
AYPGQITSNM FCAGYLEGGK DSCQGDSGGP VVCSGKLQGI VSWGSGCAQK
NKPGVYTKVC NYVSWIKQTI ASN

IVGGYTCAAN SIPYQVSLNS GSHFCGGSLI NSQWVVSAAH CYKSRIQVRL
GEHNIDVLEG NEQFINAAKI ITHPNFNGNT LDNDIMLIKL SSPATLNSRV
ATVSLPRSCA AAGTECLISG WGNTKSSGSS YPSLLQCLKA PVLSDSSCKS
SYPGQITGNM ICVGFLEGGK DSCQGDSGGP VVCNGQLQGI VSWGYGCAQK
NKPGVYTKVC NYVNWIQQTI AAN

▲$C_{1012}H_{1585}N_{279}O_{324}S_{14}$▲ (ERR 1-Dec-2020) 23,293 (for bovine β-Trypsin)
$C_{1020}H_{1597}N_{287}O_{321}S_{14}$ 23,463 (for porcine β-Trypsin) CAS RN®: 9002-07-7.▲ (USP 1-Dec-2020)

DEFINITION

Change to read:

▲▲ (USP 1-Dec-2020) Trypsin is a proteolytic enzyme ▲purified▲ (USP 1-Dec-2020) from an extract of the pancreas of healthy bovine or porcine animals. ▲▲ (USP 1-Dec-2020) When assayed as directed herein, it contains NLT ▲85 Trypsin Units/mg,▲ (USP 1-Dec-2020) calculated on the dried basis, and NLT 90.0% and NMT 110.0% of the labeled potency.

▲▲ (USP 1-Dec-2020)

IDENTIFICATION

Add the following:

▲• **A.** It meets the requirements in the *Assay*.▲ (USP 1-Dec-2020)

Add the following:

▲• **B.**

Solution A: 0.1% (v/v) phosphoric acid in water prepared as follows. To 1000 mL of water add 1 mL of 85% phosphoric acid.

Solution B: 0.1% (v/v) phosphoric acid in acetonitrile prepared as follows. To 1000 mL of acetonitrile add 1 mL of 85% phosphoric acid.

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	75	25
25	55	45
30	10	90
34	10	90
35	75	25
45	75	25

Diluent: 20 mM calcium chloride and 0.01 N hydrochloric acid, pH 2.0 ± 0.2 prepared as follows. Dissolve 2.9 g of calcium chloride dihydrate in approximately 700 mL of water, add 2.5 mL of 4 N hydrochloric acid, mix well, and dilute with water to a final volume of 1000 mL. Adjust, if necessary, with 4 N hydrochloric acid to a pH of 2.0 ± 0.2.

Standard solution

For USP Trypsin Bovine RS: Dissolve a weighed quantity of USP Trypsin Bovine RS in *Diluent* to obtain a concentration of 70 mg/mL. Brief centrifugation at 2°–8° may be necessary to remove the insoluble particles.

For USP Trypsin Recombinant Porcine RS: The target protein concentration is 70 ± 10 mg/mL. Thaw 100 µL of USP Trypsin Recombinant Porcine RS at room temperature for about 1 h and mix.

Sample solution: Dissolve a weighed quantity of Trypsin of the appropriate species in *Diluent* to obtain a concentration of 70 mg/mL. Brief centrifugation at 2°–8° may be necessary to remove the insoluble portion of the sample.

Chromatographic system

(See *Chromatography ⟨621⟩, System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm × 25-cm; 3-µm packing L1, pore size 200 Å

Temperatures

Autosampler: 5°

Column: 40°

Flow rate: 1.0 mL/min

Injection volume: 1 µL

System suitability

Sample: *Standard solution*

[Note—The relative retention times for α-trypsin and β-trypsin are 0.96 and 1.0, respectively. The retention time for the β-trypsin from bovine trypsin is approximately 11 min and from porcine trypsin is approximately 14 min.]

Suitability requirements

Resolution: NLT 1 between the α-trypsin and β-trypsin peaks

Analysis

Samples: *Standard solution* and *Sample solution*

Acceptance criteria: The retention time of the β-trypsin peak of the *Sample solution* corresponds to that of the appropriate species of the *Standard solution*.▲ (USP 1-Dec-2020)

ASSAY

Change to read:

• **Procedure**

▲**Buffer:** 100 mM Tris and 20 mM calcium chloride, pH 8.0, prepared as follows. Dissolve 1.21 g of tris(hydroxymethyl)aminomethane and 0.29 g of calcium chloride dihydrate in 100 mL of water, and adjust with 2 N hydrochloric acid to a pH of 8.0 (at 25 ± 1°).

Diluent: 20 mM calcium chloride and 0.01 N hydrochloric acid, pH 2.0 ± 0.2, prepared as follows. Dissolve 2.9 g of calcium chloride dihydrate in approximately 700 mL of water, add 2.5 mL of 4 N hydrochloric acid, mix well, and dilute with water to a final volume of 1000 mL. Adjust, if necessary, with 4 N hydrochloric acid to a pH of 2.0 ± 0.2.

Substrate stock solution: Dissolve 20 mg of carbobenzoxy-valyl-glycyl-arginine-4-nitril-anilide acetate,[1] accurately weighed, in 3.0 mL of water. [1][Note—Use a freshly prepared solution only.]

Substrate solution: Mix 28 mL of *Buffer* and 2.8 mL of *Substrate stock solution*.

Standard solution

For USP Trypsin Bovine RS: Dissolve a weighed quantity of USP Trypsin Bovine RS in *Diluent* to obtain a concentration of 2,000–2,400 Trypsin Units/mL. Prepare each *Standard solution* by diluting this solution with water using a serial dilution scheme to obtain a final concentration of approximately 0.25 Trypsin Units/mL. Prepare NLT 5 *Standard solutions* in parallel. *Assay* each *Standard solution* in duplicate.

For USP Trypsin Recombinant Porcine RS: Precool USP Trypsin Recombinant Porcine RS and water to approximately 4°. Start preparing *Standard solutions* immediately when the temperature has reached 4°. Prepare each *Standard solution* by diluting USP Trypsin Recombinant Porcine RS with water using a serial dilution scheme to obtain a final concentration of approximately 0.2 Trypsin Units/mL. Prepare NLT 5 *Standard solutions* in parallel. *Assay* each *Standard solution* in duplicate.

Sample stock solution: Dissolve a quantity of Trypsin of the appropriate species in *Diluent* to obtain a concentration of 10–20 mg/mL.

Sample solution: Dilute *Sample stock solution* with water using a serial dilution scheme to obtain a final concentration of approximately 0.25 Trypsin Units/mL. Prepare NLT 5 *Sample solutions* in parallel. *Assay* each *Sample solution* in duplicate.

[Note—Use an adjustable pipettor for each measurement and dilution operation. Use polystyrene test tubes when preparing the *Standard solution* and *Sample solution*, and use polystyrene pipet tips containing polyethylene filters to transfer samples. The pipet tip should not be wet before transfer, and each pipet tip should only be used for transferring one sample.]

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)

Mode: UV

Analytical wavelength: 405 nm

Cell: 1 cm

Temperature: 25°

Analysis

Samples: *Standard solution* and *Sample solution*

Transfer 1.10 mL of *Substrate solution* into a polystyrene semimicro cuvette, allow the temperature to stabilize, check the cuvette for the specified temperature, and wait for 10 min. Start the reaction by adding 0.020 mL of *Standard solution* or *Sample solution*. Record the absorbance for at least 5 min, and determine the change in absorbance (ΔA/min) from the linear range of the reaction.

Calculate the activity of trypsin in Trypsin Units/mg in the portion of Trypsin taken:

$$\text{Result} = \{[V_T/(\varepsilon_{405} \times V \times B)] \times (\Delta A/\text{min}) \times D\}/C$$

V_T = volume of the reaction mixture, 1.12 mL

ε_{405} = extinction coefficient for 405 nm, 10.4 ($\text{mmol}^{-1} \cdot 1\ \text{cm}^{-1}$)

V = volume of the *Standard solution* or *Sample solution*, 0.020 mL

B = absorption of cell length, 1 cm

D = dilution factor used to prepare the *Sample solution* from the *Sample stock solution*

C = concentration of Trypsin in *Sample stock solution* (mg/mL)

[Note—1 Trypsin Unit is the activity releasing the equivalent of 1 mmol/min of 4-nitril aniline from carbobenzoxy-valyl-glycyl-arginine-4-nitril-anilide acetate under the conditions of the *Assay*.]

System suitability

Samples: *Standard solution* and *Sample solution*

Suitability requirements

ΔA/min: 0.03–0.07, *Standard solution* and *Sample solution*

Average calculated activity: 80%–120% of the labeled value, *Standard solution*

Relative standard deviation: NMT 5% for the activities determined from 5 replicates, *Standard solution* and *Sample solution*

Acceptance criteria

Specific activity: NLT 85 Trypsin Units/mg on the dried basis

Labeled potency: 90.0%–110.0%▲ (USP 1-Dec-2020)

IMPURITIES

• **Residue on Ignition ⟨281⟩:** NMT 2.5%

Change to read:

• **Organic Impurities: Limit of Chymotrypsin**

▲**Monobasic potassium phosphate solution:** 9.08 mg/mL monobasic potassium phosphate

Dibasic sodium phosphate solution: 9.46 mg/mL dibasic anhydrous sodium phosphate▲ (USP 1-Dec-2020)

0.067 M phosphate buffer, pH 7.0: ▲*Monobasic potassium phosphate solution* and *Dibasic sodium phosphate solution* (38.9:61.1). Adjust dropwise, if necessary, with *Dibasic sodium phosphate solution* to a pH of 7.0.▲ (USP 1-Dec-2020)

Substrate solution: Dissolve 0.474 mg/mL of *N*-acetyl-L-tyrosine ethyl ester, suitable for use in determining chymotrypsin, in *0.067 M phosphate buffer, pH 7.0* with warming. When cool, dilute with *0.067 M phosphate buffer, pH 7.0* (1:1). [Note—The *Substrate solution* may be stored in the frozen state and used after thawing; it is important, however, to freeze immediately after preparation.]

Sample solution: ▲22 Trypsin Units/mL of Trypsin▲ (USP 1-Dec-2020) in 0.0010 N hydrochloric acid

▲**Blank solution:** Add 3 mL of water to 0.2 mL of 0.0012 N hydrochloric acid.▲ (USP 1-Dec-2020)

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy ⟨857⟩*.)

Mode: UV

Analytical wavelength: 237 nm

Cell: 1 cm

▲**Temperature:** 25°▲ (USP 1-Dec-2020)

Analysis

[Note—Conduct the test in a suitable spectrophotometer equipped to maintain a temperature of 25° ▲▲ (USP 1-Dec-2020) in the cell compartment. Determine the temperature in the reaction cell before and after the measurement of absorbance to ensure that the temperature does not change by more than ▲1.0°.▲ (USP 1-Dec-2020)]

Samples: *Sample solution* and ▲*Blank solution*▲ (USP 1-Dec-2020)

Pipet ▲3.0 mL of *Blank solution*▲ (USP 1-Dec-2020) into a 1-cm cell. Place this cell in the spectrophotometer and ▲autozero the instrument with the *Blank solution*.▲ (USP 1-Dec-2020) Pipet 200 µL of *Sample solution* into another 1-cm cell, add 3.0 mL of the *Substrate solution*, and place the cell in the spectrophotometer.

[Note—This order of addition is to be followed.]

At the time the *Substrate solution* is added, start a stopwatch, and read the absorbance at 30-s intervals for NLT 5 min. Repeat the procedure on the same dilution at least once. Absolute absorbance values are of less importance than the constancy of the rate of change of absorbance. If the rate of change does not remain constant for at least 3 min, repeat the run, and if necessary, use a lower concentration. The duplicate run at the same dilution should match the first run in rate of absorbance change. Determine the average absorbance change/min, using only the values within the 3-min portion of the curve where the rate of absorbance is constant. Plot a curve of absorbance against time.▲▲ (USP 1-Dec-2020)

Calculate the number of USP Chymotrypsin Units/mg of ▲▲ (USP 1-Dec-2020) Trypsin taken:

$$\text{Result} = (A_2 - A_1)/(F \times T \times W)$$

A_2 = absorbance straight-line initial reading

A_1 = absorbance straight-line final reading

F = chymotrypsin activity conversion factor, 0.0075/min

T = elapsed time between the initial and final readings (min)

W = weight of ▲▲ (USP 1-Dec-2020) Trypsin in the portion of solution used to determine absorbance (mg)

▲

[Note—One USP Chymotrypsin Unit is the activity causing a change in absorbance of 0.0075/min under the conditions specified in this test.]

▲ (USP 1-Dec-2020)

Acceptance criteria: NMT 50 USP Chymotrypsin Units/▲85 Trypsin Units,▲ (USP 1-Dec-2020) indicating the presence of NMT approximately 5% ▲(w/w)▲ (USP 1-Dec-2020) of chymotrypsin

SPECIFIC TESTS

• **Microbial Enumeration Tests ⟨61⟩** and **Tests for Specified Microorganisms ⟨62⟩:** It meets the requirements of the tests for absence of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Salmonella* species.

Change to read:

• **Solubility Test:** An amount, equivalent to ▲17,000 Trypsin Units,▲ (USP 1-Dec-2020) is soluble in 10 mL of water and in 10 mL of saline TS.

• **Loss on Drying ⟨731⟩**

Analysis: Dry under vacuum at 60° for 4 h.

Acceptance criteria: NMT 5.0%

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight containers, and avoid exposure to excessive heat.

Add the following:

▲• **Labeling:** Label it to indicate that it has been prepared from bovine or porcine pancreas.▲ (USP 1-Dec-2020)

Change to read:

• **USP Reference Standards ⟨11⟩**

▲ USP Trypsin Bovine RS

USP Trypsin Recombinant Porcine RS▲ (USP 1-Dec-2020)

[1] A suitable carbobenzoxy-valyl-glycyl-arginine-4-nitril-anilide acetate is Chromozym TRY from Roche Applied Science (catalog #10378496103) or equivalent.

Auxiliary Information - Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
TRYPSIN	Julie Zhang Associate Science & Standards Liaison	BIO2 Biologics Monographs 2 - Proteins
REFERENCE STANDARD SUPPORT	RS Technical Services RSTECH@usp.org	BIO2 Biologics Monographs 2 - Proteins

Chromatographic Database Information: Chromatographic Database

Most Recently Appeared In:
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