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Pancrelipase

» Pancrelipase is a substance containing enzymes, principally lipase, with amylase and protease, obtained from the pancreas of the hog, *Sus scrofa* Linné var. *domesticus* Gray (Fam. Suidae). It contains, in each mg, not less than 24 USP Units of lipase activity, not less than 100 USP Units of amylase activity, and not less than 100 USP Units of protease activity.

[Note—One USP Unit of amylase activity is contained in the amount of pancrelipase that decomposes starch at an initial rate such that 0.16 µEq of glycosidic linkage is hydrolyzed per minute under the conditions of the *Assay for amylase activity*.]

One USP Unit of lipase activity is contained in the amount of pancrelipase that liberates 1.0 µEq of acid per minute at pH 9.0 and 37° under the conditions of the *Assay for lipase activity*.

One USP Unit of protease activity is contained in the amount of pancrelipase that under the conditions of the *Assay for protease activity*, hydrolyzes casein at an initial rate such that there is liberated per minute an amount of peptides not precipitated by trichloroacetic acid that gives the same absorbance at 280 nm as 15 nmol of tyrosine.

Packaging and storage—Preserve in tight containers, preferably at a temperature not exceeding 25°.

Labeling—Label it to indicate lipase activity in USP Units.

USP Reference standards ⟨11⟩—

- USP Bile Salts RS
- USP Pancreatin Amylase and Protease RS
- USP Pancreatin Lipase RS

Microbial enumeration tests ⟨61⟩ **and Tests for specified microorganisms** ⟨62⟩—It meets the requirements of the tests for absence of *Salmonella* species and *Escherichia coli*.

Loss on drying ⟨731⟩—Dry it in vacuum at 60° for 4 hours: it loses not more than 5.0% of its weight.

Fat—Place 2.0 g of Pancrelipase in a flask of about 50-mL capacity, add 20 mL of ether, insert the stopper, and set aside for 2 hours, mixing by rotating at frequent intervals. Decant the supernatant ether by means of a guiding rod into a plain filter about 7 cm in diameter, previously moistened with ether, and collect the filtrate in a tared beaker. Repeat the extraction with a 10-mL portion of ether, then with another 10-mL portion of ether, transfer the ether and the remainder of the Pancrelipase to the filter. Allow to drain, evaporate the ether spontaneously, and dry the residue at 105° for 2 hours: the residue of fat weighs not more than 100 mg (5.0%).

Assay for amylase activity (*Starch digestive power*)—

pH 6.8 Phosphate buffer—On the day of use, dissolve 13.6 g of monobasic potassium phosphate in water to make 500 mL of solution. Dissolve 14.2 g of anhydrous dibasic sodium phosphate in water to make 500 mL of solution. Mix 51 mL of the monobasic potassium phosphate solution with 49 mL of the dibasic sodium phosphate solution. If necessary, adjust by the dropwise addition of the appropriate solution to a pH of 6.8.

Substrate solution—On the day of use, stir a portion of purified soluble starch equivalent to 2.0 g of dried substance with 10 mL of water, and add this mixture to 160 mL of boiling water. Rinse the beaker with 10 mL of water, add it to the hot solution, and heat to boiling, with continuous mixing. Cool to room temperature, and add water to make 200 mL.

Standard preparation—Weigh accurately about 20 mg of USP Pancreatin Amylase and Protease RS into a suitable mortar. Add about 30 mL of *pH 6.8 Phosphate buffer*, and triturate for 5 to 10 minutes. Transfer the mixture with the aid of *pH 6.8 Phosphate buffer* to a 50-mL volumetric flask, dilute with *pH 6.8 phosphate buffer* to volume, and mix. Calculate the activity, in USP Units of amylase activity per mL, of the resulting solution from the declared potency on the label of the Reference Standard.

Assay preparation—For Pancrelipase having about 4 times the amylase activity of the USP Pancreatin Amylase and Protease RS, weigh accurately about 10 mg of Pancrelipase into a suitable mortar. [Note—For Pancrelipase having a different amylase activity, weigh accurately the amount necessary to obtain an *Assay preparation* having amylase activity per mL corresponding approximately to that of the *Standard preparation*.] Add about 3 mL of *pH 6.8 Phosphate buffer*, and triturate for 5 to 10 minutes. Transfer the mixture with the aid of *pH 6.8 Phosphate buffer* to a 100-mL volumetric flask, dilute with *pH 6.8 Phosphate buffer* to volume, and mix.

Procedure—Prepare four stoppered, 250-mL conical flasks, and mark them *S*, *U*, *BS*, and *BU*. Pipet into each flask 25 mL of *Substrate solution*, 10 mL of *pH 6.8 Phosphate buffer*, and 1 mL of sodium chloride solution (11.7 in 1000), insert the stoppers, and mix. Place the flasks in a water bath maintained at 25 ± 0.1°, and allow them to equilibrate. To flasks *BU* and *BS* add 2 mL of 1 N hydrochloric acid, mix, and return the

flasks to the water bath. To flasks *U* and *BU* add 1.0-mL portions of *Assay preparation*, and to flasks *S* and *BS* add 1.0 mL of *Standard preparation*. Mix each, and return the flasks to the water bath. After 10 minutes, accurately timed from the addition of the enzyme, add 2-mL portions of 1 N hydrochloric acid to flasks *S* and *U*, and mix. To each flask, with continuous stirring, add 10.0 mL of 0.1 N iodine VS, and immediately add 45 mL of 0.1 N sodium hydroxide. Place the flasks in the dark at a temperature between 15° and 25° for 15 minutes. To each flask add 4 mL of 2 N sulfuric acid, and titrate with 0.1 N sodium thiosulfate VS to the disappearance of the blue color. Calculate the amylase activity, in USP Units per mg, of the Pancrelipase taken by the formula:

$$100(C_S/W_U)(V_{BU} - V_U)/(V_{BS} - V_S)$$

in which C_S is the amylase activity of the *Standard preparation*, in USP Units per mL, W_U is the amount, in mg, of Pancrelipase taken, and V_U, V_S, V_{BU}, and V_{BS} are the volumes, in mL, of 0.1 N sodium thiosulfate consumed in the titration of the solutions in flasks *U*, *S*, *BU*, and *BS*, respectively.

Assay for lipase activity (*Fat digestive power*)—

Acacia solution—Centrifuge a solution of acacia (1 in 10) until clear. Use only the clear solution.

Olive oil substrate—Combine 165 mL of *Acacia solution*, 20 mL of olive oil, and 15 g of crushed ice in the cup of an electric blender. Cool the mixture in an ice bath to 5°, and homogenize at high speed for 15 minutes, intermittently cooling in an ice bath to prevent the temperature from exceeding 30°.

Test for suitability of mixing as follows. Place a drop of the homogenate on a microscope slide, and gently press a cover slide in place to spread the liquid. Examine the entire field under high power (43× objective lens and 5× ocular), using an eyepiece equipped with a calibrated micrometer. The substrate is satisfactory if 90% of the particles do not exceed 2 µm in diameter and none exceeds 10 µm in diameter.

Buffer solution—Dissolve 60 mg of tris(hydroxymethyl) aminomethane and 234 mg of sodium chloride in water to make 100 mL.

Bile salts solution—Prepare a solution to contain 80.0 mg of USP Bile Salts RS in each mL.

Standard test dilution—Suspend about 200 mg of USP Pancreatin Lipase RS, accurately weighed, in about 3 mL of cold water in a mortar, triturate for 10 minutes, and add cold water to a volume necessary to produce a concentration of 8 to 16 USP Units of lipase activity per mL, based upon the declared potency on the label of the Reference Standard. Maintain the suspension at 4°, and mix before using. For each determination withdraw 5 to 10 mL of the cold suspension, and allow the temperature to rise to 20° before pipeting the exact volume.

Assay test dilution—Suspend about 200 mg of Pancrelipase, accurately weighed, in about 3 mL of cold water in a mortar, triturate for 10 minutes, and add cold water to a volume necessary to produce a concentration of 8 to 16 USP Units of lipase activity per mL, based upon the estimated potency of the test material. Maintain the suspension at 4°, and mix before using. For each determination withdraw 5 to 10 mL of the cold suspension, and allow the temperature to rise to 20° before pipeting the exact volume.

Procedure—Mix 10.0 mL of *Olive oil substrate*, 8.0 mL of *Buffer solution*, 2.0 mL of *Bile salts solution*, and 9.0 mL of water in a jacketed glass vessel of about 50-mL capacity, the outer chamber of which is connected to a thermostatically controlled water bath. Cover the mixture, and stir continuously with a mechanical stirring device. With the mixture maintained at a temperature of 37 ± 0.1°, add 0.1 N sodium hydroxide VS, from a microburet inserted through an opening in the cover, to adjust the pH to 9.20 potentiometrically using a calomel-glass electrode system. Add 1.0 mL of *Assay test dilution*, and then continue adding the 0.1 N sodium hydroxide VS for 5 minutes to maintain the pH at 9.0. Determine the volume of 0.1 N sodium hydroxide VS added after each minute.

In the same manner titrate 1.0 mL of *Standard test dilution*.

Calculation of potency—Plot the volume of 0.1 N sodium hydroxide VS titrated against time. Using only the points which fall on the straight-line segment of the curve, calculate the mean acidity released per minute by the test specimen and the Standard. Taking into consideration the dilution factors, calculate the lipase activity, in USP Units, of the Pancrelipase taken by comparison to the activity of the Reference Standard, using the lipase activity stated on the label of USP Pancreatin Lipase RS.

Assay for protease activity (*Casein digestive power*)—

Casein substrate—Place 1.25 g of finely powdered casein in a 100-mL conical flask containing 5 mL of water, shake to form a suspension, add 10 mL of 0.1 N sodium hydroxide, shake for 1 minute, add 50 mL of water, and shake for about 1 hour to dissolve the casein. If necessary, adjust to a pH of about 8, using 1 N sodium hydroxide or 1 N hydrochloric acid. Transfer the solution quantitatively to a 100-mL volumetric flask, dilute with water to volume, and mix. Use this substrate on the day it is prepared.

Buffer solution—Dissolve 6.8 g of monobasic potassium phosphate and 1.8 g of sodium hydroxide in 950 mL of water in a 1000-mL volumetric flask, adjust to a pH of 7.5 ± 0.2, using 0.2 N sodium hydroxide, dilute with water to volume, and mix. Store this solution in a refrigerator.

Trichloroacetic acid solution—Dissolve 50 g of trichloroacetic acid in 1000 mL of water. This solution may be stored at room temperature.

Filter paper—Determine the suitability of the filter paper by filtering a 5-mL portion of *Trichloroacetic acid solution* through the paper and measuring the absorbance of the filtrate at 280 nm, using an unfiltered portion of the same *Trichloroacetic acid solution* as the blank: the absorbance is not more than 0.04. If the absorbance is more than 0.04, wash the filter paper repeatedly with *Trichloroacetic acid solution* until the absorbance of the filtrate, determined as above, is not more than 0.04.

Standard test dilution—Add about 100 mg of USP Pancreatin Amylase and Protease RS, accurately weighed, to 100.0 mL of *Buffer solution*, and mix by shaking intermittently at room temperature for about 25 minutes. Dilute quantitatively with *Buffer solution* to produce a concentration of about 2.5 USP Units of protease activity per mL, based upon the declared potency on the label of the Reference Standard.

Assay test dilution—Weigh accurately about 100 mg of Pancrelipase into a suitable mortar. Add about 3 mL of *Buffer solution*, and triturate for 5 to 10 minutes. Transfer the mixture with the aid of *Buffer solution* to a 100-mL volumetric flask, dilute with *Buffer solution* to volume, and mix. Dilute quantitatively with *Buffer solution* to obtain a dilution that corresponds in activity to the *Standard test dilution*.

Procedure—Label test tubes in duplicate S_1, S_2, and S_3 for the standard series, and U for the sample. Pipet into tubes S_1 2.0 mL, into S_2 and U 1.5 mL, and into S_3 1.0 mL of *Buffer solution*. Pipet into tubes S_1 1.0 mL, into S_2 1.5 mL, and into S_3 2.0 mL of the *Standard test dilution*. Pipet into tubes U 1.5 mL of the *Assay test dilution*. Pipet into one tube each of S_1, S_2, S_3, and U 5.0 mL of *Trichloroacetic acid solution*, and mix. Designate these tubes as S_{1B}, S_{2B}, S_{3B}, and U_B, respectively. Prepare a blank by mixing 3 mL of *Buffer solution* and 5 mL of *Trichloroacetic acid solution* in a separate test tube marked *B*. Place all the tubes in a 40° water bath, insert a glass stirring rod into each tube, and allow for temperature equilibration. At zero time, add to each tube, at timed intervals, 2.0 mL of the *Casein substrate*, preheated to the bath temperature, and mix. Sixty minutes, accurately timed, after the addition of the *Casein substrate* stop the reaction in tubes S_1, S_2, S_3, and U by adding 5.0 mL of *Trichloroacetic acid solution* at the corresponding time intervals, stir, and remove all the tubes from the bath. Allow to stand at room temperature for 10 minutes for complete protein precipitation, and filter. The filtrates must be free from haze. Determine the absorbances of the filtrates, in 1-cm cells, at 280 nm, with a suitable spectrophotometer, using the filtrate from the blank (tube *B*) to set the instrument.

Calculation of potency—Correct the absorbance values for the filtrates from tubes S_1, S_2, and S_3 by subtracting the absorbance values for the filtrates from tubes S_{1B}, S_{2B}, and S_{3B}, respectively, and plot the corrected absorbance values against the corresponding volumes of the *Standard test dilution* used. From the curve, using the corrected absorbance value ($U - U_B$) for the Pancrelipase taken, and taking into consideration the dilution factors, calculate the protease activity, in USP Units, of the Pancrelipase taken by comparison with that of the Standard, using the protease activity stated on the label of USP Pancreatin Amylase and Protease RS.

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

Topic/Question	Contact	Expert Committee
PANCRELIPASE	Jennifer Tong Sun Senior Scientist II	BIO2 Biologics Monographs 2 - Proteins
REFERENCE STANDARD SUPPORT	RS Technical Services RSTECH@usp.org	BIO2 Biologics Monographs 2 - Proteins

Chromatographic Database Information: Chromatographic Database

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