

Status: Currently Official on 16-Feb-2025
 Official Date: Official as of 01-Aug-2013
 Document Type: Reagents
 DocId: GUID-BCC8FF69-EF08-4594-B543-6CEB61E7F0EE_1_en-US
 DOI: https://doi.org/10.31003/USPNF_R3673_01_01
 DOI Ref: tp3gm

© 2025 USPC
 Do not distribute

Pectate Lyase

CAS RN[®]: 9015-75-2.—An enzyme obtained from *Aspergillus sp.* Light brown, viscous liquid. Specific gravity is about 1.5. It is readily soluble in water. It is supplied at approximately 14 units per mL at pH 8.0 in Tris-HCl buffer [50 mM of Tris(hydroxymethyl)aminomethane containing 1 mM of CaCl₂, pH 8.0] in a solution of 50% glycerol and 0.02% sodium azide. One unit is defined as the enzyme activity that produces 1 μmol of unsaturated product per minute.

Activity

Pectin solution: Transfer a quantity of Pectin, equivalent to 0.05 g on the dried basis, to a 100-mL volumetric flask.

[NOTE—Pectin has a molecular weight of 103,000 Da; its degree of esterification (percentage of galacturonic acid groups substituted with methyl) is 12.]

Moisten with 0.1 mL of 2-propanol. Add 50 mL of water to the flask, and mix the solution with a magnetic stirrer. Use 0.5 N sodium hydroxide to adjust the solution to a pH of 12. Stop the stirrer, and allow the solution to stand undisturbed at room temperature for 15 minutes. Adjust the solution with 0.5 N hydrochloric acid to a pH of 8.0. Dilute with water to volume.

Tris buffer solution: Transfer 6.055 g of Tris(hydroxymethyl)aminomethane and 0.147 g of calcium chloride to a 1000-mL volumetric flask containing 950 mL of water, and mix. Adjust the solution with 1 N hydrochloric acid to a pH of 8.0. Dilute with water to volume.

Diluted pectate lyase: Transfer 0.5 mL of Pectate Lyase to a 50-mL volumetric flask, dilute with *Tris buffer solution* to volume, and mix.

Procedure: Add the solutions set forth in the table below to quartz cuvettes.

Label	Tris Buffer Solution (mL)	Pectin Solution (mL)	Diluted Pectate Lyase (mL)	Water (mL)
<i>Enzyme blank</i>	0.5	1.0	0	1.0
<i>Test blank</i>	0.5	0	0.5	1.5
<i>Test solution</i>	0.5	1.0	0.5	0.5

Perform the test on the solutions so obtained, using a suitable UV-Vis spectrophotometer (see [Ultraviolet-Visible Spectroscopy \(857\)](#)) and using water as the blank. Mix the solutions well at time 0, and immediately measure the absorbances at 235 nm. Record the value for the *Enzyme blank*, A_{0-EB} ; for the *Test blank*, A_{0-TB} ; and for the *Test solution*, A_{0-TS} . After incubation at room temperature for 30 minutes, determine the absorbance again at 235 nm for the *Enzyme blank*, A_{30-EB} ; for the *Test blank*, A_{30-TB} ; and for the *Test solution*, A_{30-TS} . One unit is defined as the enzymatic activity that produces 1 μmol of unsaturated product from pectin per minute. Calculate the Pectate Lyase activity, in units per mL, using the following formula:

$$50(10^3)[(A_{30-TS} - A_{30-EB} - A_{30-TB}) - (A_{0-TS} - A_{0-EB} - A_{0-TB})]/30E_{235}L$$

in which 50 is the volume, in mL, of *Diluted pectate lyase*; 10^3 is the unit conversion factor; 30 is the time, in minutes, of the reaction; E_{235} is the molar extinction coefficient, in $M^{-1} cm^{-1}$, of the reaction product ($4600 M^{-1} cm^{-1}$); and L is the path length, in cm, of the reaction cuvette (1 cm). Alternatively, these solutions, after being mixed in the cuvettes, can be immediately measured at 235 nm continuously in a recording UV-Vis spectrophotometer set up for kinetic assays. The result is obtained by correcting the blank determination, using the *Enzyme blank* and the *Test blank*.

Auxiliary Information - Please [check for your question in the FAQs](#) before contacting USP.

Topic/Question	Contact	Expert Committee
PECTATE LYASE	Margareth R.C. Marques Principal Scientific Liaison	HDQ Headquarters

Most Recently Appeared In:

Pharmacopeial Forum: Volume No. PF 38(3)

Current DocID: [GUID-BCC8FF69-EF08-4594-B543-6CEB61E7F0EE_1_en-US](#)

DOI: https://doi.org/10.31003/USPNF_R3673_01_01

DOI ref: [tp3gm](#)

OFFICIAL