

Status: Currently Official on 14-Feb-2025  
 Official Date: Official as of 01-Dec-2023  
 Document Type: Reagents  
 DocId: GUID-6B7922AD-798A-4DA1-B54B-9DD6EE2123A\_4\_en-US  
 DOI: [https://doi.org/10.31003/USPNF\\_R1299\\_04\\_01](https://doi.org/10.31003/USPNF_R1299_04_01)  
 DOI Ref: 9j2gj

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## Bromelain

CAS RN<sup>®</sup>: 9001-00-7.

A glycoprotein that is highly active thiol proteinase. It is found in the leaves and stems of the pineapple plant. Yellowish-white to tan powder.

### • ACTIVITY DETERMINATION

**pH 4.5 water:** Adjust with [0.1 N hydrochloric acid](#) to a pH of 4.5.

**Gelatin substrate:** Dissolve 25 g of gelatin in 375 mL of hot [water](#). Bring to a boil. Cool to 45°. Adjust the pH to 4.5 with [0.1 N hydrochloric acid](#). Dilute with *pH 4.5 water* to 500 mL. Keep it at 45°. This substrate should be prepared fresh daily.

**Buffer solution:** Add 150 g of [sodium chloride](#) to 700 mL of *pH 4.5 water*, stir to dissolve, then add 5.7 mL of [acetic acid](#). Adjust with 50% [sodium hydroxide](#) to a pH of 4.5, if necessary. Dilute to 1 L.

**3% Hydrogen peroxide solution:** Transfer 2.5 mL of hydrogen peroxide to a 25-mL volumetric flask. Dilute with *pH 4.5 water* to volume.

**pH 9.0 formaldehyde solution:** Adjust a 100-mL [formaldehyde solution](#) to a pH of 9.0 with [0.1 N sodium hydroxide VS](#).

**Bromelain preparation:** Weigh 100 mg of bromelain with a theoretical activity of 2400 GDU/g. If the sample activity differs by more than 10% from 2400 GDU/g, determine the sample weight:

$$\text{mg of sample} = (2400 \times 100) / \text{theoretical activity}$$

Transfer the sample to a 50-mL volumetric flask. Add 8.3 mL of *Buffer solution*. Let stand for 30 min at room temperature. Dilute with *pH 4.5 water* to volume. Add a small stir bar and stir for 10–15 min, ▲resulting in a suspension as bromelain will not dissolve.▲ (USP 1-Dec-2023)

**Procedure:** Transfer 25 mL of *Gelatin substrate* to each of two 100-mL beakers containing stir bars and place them in a water bath at 45° for 5 min. One beaker is for the *Test solution* and the other for the *Blank solution*.

**Test solution:** Add 1.0 mL of *Bromelain preparation* into the beaker, start timing, and swirl. After exactly 20 min of incubation at 45°, add 0.1 mL of 3% *Hydrogen peroxide solution*, and swirl. Incubate for an additional 5 min. Remove the beaker from the water bath and, with constant stirring, insert the pH electrode. Record the pH after 10 s (initial pH). Adjust with [0.1 N sodium hydroxide VS](#) to a pH of 6.0. [NOTE –When adjusting the pH to 6.0 be cautious at pH 5.8; the pH increases slowly but minute additions of sodium hydroxide at this point will significantly increase the pH.] Add 10 mL of *pH 9.0 formaldehyde solution* with constant stirring. Titrate to a pH of 9.0 with [0.1 N sodium hydroxide VS](#). Record the volume of titrant used. This is the test titer, *T*.

**Blank solution:** Run concurrently with the *Test solution* by starting the *Blank solution* determination 12 min after the *Test solution* is started. Add 0.1 mL of 3% *Hydrogen peroxide solution* to the *Blank solution* beaker, and swirl. After exactly 20 min of incubation at 45°, add 1.0 mL of *Bromelain preparation*, and swirl. Incubate for an additional 15 min. Remove the beaker from the water bath and, with constant stirring, insert the pH electrode. Record the pH after 10 s (initial pH). Adjust with [0.1 N sodium hydroxide](#) to a pH of 6.0. See the *Note* under *Test solution*. Add 10 mL of *pH 9.0 formaldehyde solution* with constant stirring. Titrate to a pH of 9.0 with [0.1 N sodium hydroxide VS](#). Record the volume of titrant used. This is the blank titer, *B*.

**Calculation:** 1 Gelatin Digestion Unit (GDU) is the amount of enzyme that, after 20 min of digestion at 45°, will liberate 1 mg of amino nitrogen from a standard gelatin solution at a pH of 4.5.

$$\text{GDU/g} = (T - B) \times 14 \times N \times (50/W)$$

*T* = mL of 0.1 N sodium hydroxide used with the *Test solution*

*B* = mL of 0.1 N sodium hydroxide used with the *Blank solution*

*N* = actual normality of 0.1 N sodium hydroxide VS from standardization

*W* = weight of bromelain taken (g)

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

Topic/Question	Contact	Expert Committee
BROMELAIN	<a href="#">Margareth R.C. Marques</a> Principal Scientific Liaison	HDQ Headquarters

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Pharmacopeial Forum: Volume No. 48(4)

**Current DocID: GUID-6B7922AD-798A-4DA1-B54B-9DDD6EE2123A\_4\_en-US****DOI: [https://doi.org/10.31003/USPNF\\_R1299\\_04\\_01](https://doi.org/10.31003/USPNF_R1299_04_01)****DOI ref: [9j2gi](#)**

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