

Product Information

Chymase Assay Kit

Catalog Number **CS1140**
Storage Temperature $-20\text{ }^{\circ}\text{C}$

TECHNICAL BULLETIN

Product Description

Chymase (mast cell protease I, skeletal muscle protease, skin chymotryptic proteinase, or mast cell serine proteinase) is a chymotrypsin-like serine proteinase present in the secretory granules of mast cells.¹ This enzyme hydrolyzes proteins at their C-terminus after aromatic amino acids such as Phe, Tyr, and Trp. Its physiological substrate is angiotensin I, which upon cleavage by chymase yields angiotensin II in the final step of the renin-angiotensin system.^{2,3} Angiotensin II has an important role in hypertension as well as other pathophysiological conditions such as cardiac hypertrophy, heart failure, atherosclerosis, and glomerulosclerosis in humans.⁴ In addition, chymase is known to promote mast cell degranulation and plays a role in airway secretions and in atopic or allergic inflammation of the skin. Moreover, in the cardiovascular system, chymase is known to provoke cardiomyopathy and vascular proliferation. Thus, chymase is a target for different inhibitors that could be administered as anti-asthma or anti-allergic drugs as well as treatment against cardiovascular diseases.^{5,6}

The kit offers all reagents required for a fast and easy measurement of purified chymase activity for screening of potential inhibitors/activators. Two chymotrypsin-like substrates are supplied for convenience in the kit. One is N-Succinyl-Ala-Ala-Pro-Phe *p*-nitroanilide, a colorimetric substrate yielding a product with absorbance at 405 nm. The second substrate is N-benzoyl-L-tyrosine ethyl ester (BTEE), which for historical reasons, is more commonly used to define the enzyme specific activity. However, it is less user-friendly due to its low solubility and the fact that the absorbance of the reaction product is detected at 256 nm, requiring a UV spectrophotometer. The kit also contains an inhibitor (Chymostatin, chymotrypsin-like inhibitor) for use as a control in inhibition reactions.

Components

The kit contains sufficient reagents for 150 assays of 100 μL or 30 assays of 1 mL.

Assay Buffer Catalog Number A9606	15 mL
Chymase Catalog Number C8118	50 μg
Substrate A (N-Succinyl-Ala-Ala-Pro-Phe <i>p</i> -nitroanilide) Catalog Number S0448	1 mL
Substrate B (BTEE) Catalog Number S0573	3 mL
Inhibitor Solution (Chymostatin) Catalog Number I0285	200 μL

Reagents and Equipment Required but Not Provided

- 1 mL spectrophotometer quartz cuvette (Cat. No. S10SM), or 96 well plate (Cat. No. M9410, when using Substrate A) or UV 96 well plate (Cat. No. CLS3635, when using Substrate B)
- Temperature controlled spectrophotometer or plate reader suitable for measuring absorbance at 405 nm (Substrate A) or 256 nm (Substrate B)
- DMSO (Cat. No. D8418) as a control for the inhibition reaction when using the supplied Inhibitor Solution
- Ethanol, absolute (Cat. No. 459828 or equivalent) when using substrate B in 1 mL reactions

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

55% Ethanol Solution - Prepare 10 mL of 55% (v/v) Ethanol Solution by mixing 5.5 mL of ethanol with 4.5 mL of water. Keep the mixture at room temperature until needed (For **1 mL** assays using Substrate **B** only).

Storage/Stability

The kit is shipped on dry ice and storage at $-20\text{ }^{\circ}\text{C}$ is recommended. Upon first opening, it is recommended to store the kit components in working aliquots.

Procedures

Notes: Use ultrapure water (17 M Ω /cm or equivalent).

Testing inhibitor/regulator solutions not supplied in this kit requires verification that the solvent does not interfere with the enzymatic assay. The volume of the inhibitor/regulator solutions can be increased at the expense of the water volume used in the assay.

When following these procedures, an inhibition of $\geq 50\%$ in chymase activity is considered satisfactory.

I. Substrate A Assays

The enzyme (Chymase, Cat. No. C8118) should be kept on ice while setting up the reaction. After thawing and while setting up the reaction, Substrate A (Cat. No. S0448) and the Inhibitor Solution (Cat. No. I0285) should be kept at room temperature.

A. 1 mL Assay

Prior to the assay - Warm ultrapure water (~ 1 mL per assay) and the Assay Buffer (Cat. No. A9606) to $37\text{ }^{\circ}\text{C}$.

Set the spectrophotometer absorbance wavelength at 405 nm and the temperature at $37\text{ }^{\circ}\text{C}$.

Table 1.

Reaction Scheme for 1 mL Assay with Substrate A

Test	Water (μL)	Assay Buffer (μL)	Substrate A (μL)	Inhibitor solution or DMSO (μL)	Chymase*
Activity	635	330	30	3 (DMSO)	1 μg (2.5-5 μL)
Inhibition	635	330	30	3 (Inhibitor)	1 μg (2.5-5 μL)

* Dependent on lot specific concentration

- Add the reagents to a 1 mL quartz cuvette in the following order (see Table 1):
 - 635 μL of water
 - 330 μL of Assay Buffer (Cat. No. A9606)
 - 30 μL of Substrate A (Cat. No. S0448)
 - 3 μL of DMSO (Cat. No. D8418) to the activity reaction or 3 μL of Inhibitor Solution (Cat. No. I0285) to the inhibition reaction
- Mix by inversion and equilibrate to $37\text{ }^{\circ}\text{C}$ for 1-2 minutes in the spectrophotometer chamber.
- Before adding the enzyme, blank the spectrophotometer (i.e., set reference) using the mixture of step 2.
- Add $\sim 1\text{ }\mu\text{g}$ of Chymase (Cat. No. C8118).
- Mix by inversion and immediately record the increase in absorbance at 405 nm for 1 minute ($\Delta A_{405}/\text{min}$).

B. 100 μL Assay

Prior to the assay - Warm ultrapure water (~ 0.1 mL per assay) and the Assay Buffer (Cat. No. A9606) to $37\text{ }^{\circ}\text{C}$.

Set the plate reader absorbance wavelength at 405 nm and set the temperature at $37\text{ }^{\circ}\text{C}$. Set a kinetic program for 10 minutes reading the absorbance every 20 seconds.

Table 2.

Reaction Scheme for 100 μL Assay with Substrate A

Test	Water (μL)	Assay Buffer (μL)	Substrate A (μL)	Inhibitor solution or DMSO (μL)	Chymase*
Activity	62	33	3	0.5 (DMSO)	0.3 μg (1-1.5 μL)
Inhibition	62	33	3	0.5 (Inhibitor)	0.3 μg (1-1.5 μL)

* Dependent on lot specific concentration

- Add the reagents to each well in the following order (see Table 2):
 - 62 μL of water
 - 33 μL of Assay Buffer (Cat. No. A9606)
 - 3 μL of Substrate A (Cat. No. S0448)
 - 0.5 μL of DMSO (Cat. No. D8418) to the activity reaction or 0.5 μL of Inhibitor Solution (Cat. No. I0285) to the inhibition reaction.
- Mix well and equilibrate to $37\text{ }^{\circ}\text{C}$ in the plate reader chamber (1-2 minutes).

3. Add ~0.3 µg of Chymase (Cat. No. C8118). Mix before the first reading for 3 seconds at maximal intensity.
4. Record the kinetics of the increase in absorbance at 405 nm (consider time zero as the reaction blank).

II. Substrate B Assays

The enzyme (Chymase, Cat. No. C8118) and Substrate B (Cat. No. S0573) should be kept on ice while setting up the reaction. After thawing and while setting up the reaction, the Inhibitor Solution (Cat. No. I0285) should be kept at room temperature.

Note: Substrate B (BTEE) contains 55% ethanol, which appears to interfere with the inhibition of the supplied Inhibitor Solution (Chymostatin). Therefore, when setting an inhibition reaction using the supplied Inhibitor Solution, it is very important to follow the following procedure and to allow inhibitor/enzyme interaction prior to adding the ethanol containing Substrate B to the system.

A. 1 mL Assay

Prior to the assay - Dilute Substrate B (Cat. No. S0573) 10-fold in 55% Ethanol Solution (e.g., dilute 100 µL of Substrate B in 900 µL of 55% Ethanol Solution). While setting up the reaction keep the diluted substrate at room temperature and place the stock back on ice. Set the spectrophotometer absorbance wavelength at 256 nm and the temperature at 25 °C.

Table 3.

Reaction Scheme for 1 mL Assay with Substrate B

Sample	Water (µL)	Assay Buffer (µL)	Inhibitor solution or DMSO (µL)	Chymase*	Substrate B diluted 10-fold (µL)
Activity	360	330	3 (DMSO)	1 µg (2.5-5 µL)	300
Inhibition	360	330	3 (Inhibitor)	1 µg (2.5-5 µL)	300

* Dependent on lot specific concentration

1. Add the reagents to a 1 mL quartz cuvette in the following order (see Table 3):
 - a. 360 µL of H₂O
 - b. 330 µL of Assay Buffer (Cat. No. A9606)
 - c. 3 µL of DMSO (Cat. No. D8418) to the activity reaction or 3 µL of Inhibitor Solution (Cat. No. I0285) to the inhibition reaction. Mix well by inversion.
 - d. Add ~1 µg of Chymase (Cat. No. C8118).

2. Mix by inversion and incubate at 25 °C for 1–2 minutes in the spectrophotometer chamber.
3. Before adding the 10-fold diluted Substrate B, blank the spectrophotometer (i.e., set reference) using the mixture of step 2.
4. Add 300 µL of 10-fold diluted Substrate B.
5. Mix by inversion and immediately record the increase in absorbance at 256 nm for 1 minute ($\Delta A_{256}/\text{min}$).

B. 100 µL Assay

Prior to the assay - Set the Plate Reader absorbance wavelength at 256 nm and the temperature at 25 °C. Set a kinetic program for 5 minutes reading the absorbance every 20 seconds.

Table 4.

Reaction Scheme for 100 µL Assay with Substrate B

Sample	Water (µL)	Inhibitor solution or DMSO (µL)	Chymase*	Assay Buffer (µL)	Substrate B (µL)
Activity	36	0.5 (DMSO)	0.3 µg (1-1.5 µL)	33	30
Inhibition	36	0.5 (Inhibitor)	0.3 µg (1-1.5 µL)	33	30

* Dependent on lot specific concentration

1. Add the reagents to each well in the following order (see Table 4):
 - a. 36 µL of H₂O
 - b. 0.5 µL of DMSO (Cat. No. D8418) to the activity reaction or 0.5 µL of Inhibitor Solution (Cat. No. I0285) to the Inhibition reaction.
 - c. Add ~0.3 µg of Chymase (Cat. No. C8118) to the activity and the inhibition reactions.
2. Mix for 1–2 minutes using a horizontal shaker and incubate at 25 °C for 1–2 minutes in the plate reader chamber.
3. While incubating, in a separate microtube, mix 33 µL of Assay Buffer (Cat. No. A9606) with 30 µL of Substrate B (Cat. No. S0573) for each reaction.
4. Add 63 µL of mixture (step 3) to each well from step 2. Allow mixing before first read for 3 seconds at maximal intensity.
5. Record the kinetics of the increase in absorbance at 256 nm (consider time zero as the reaction blank).

Calculation

Specific Activity - The specific activity of chymase is defined by its ability to hydrolyze BTEE at pH 7.8 and 25 °C.

Unit definition: One unit hydrolyzes one μ mole of BTEE per minute at pH 7.8 and 25 °C.

For specific activity determination the 1 mL assay reaction is recommended in order to achieve maximal accuracy.

The specific activity can be calculated for the 1 mL assay from the following equation:

$$\text{Units/mgP} = \frac{\Delta A_{256}/\text{min} \times V}{0.964 \times \text{mgP}}$$

where:

$\Delta A_{256}/\text{min}$ = The increase in absorbance per minute in the linear range of the reaction

0.964 = Millimolar extinction coefficient of BTEE at 256 nm

mgP = The amount of protein in the reaction mixture in mg

V = The volume of the reaction in mL (1 mL)

References

1. IUBMB Enzyme Nomenclature.
2. Suzuki, T., et al., Recombinant human chymase produced by silkworm-baculovirus expression system: its application for a chymase detection kit. *Jpn. J. Pharmacol.*, **90**, 210-213 (2002).
3. Takai, S., et al., Chymase-dependent angiotensin II formation in human vascular tissue. *Circulation*, **100**, 654-658 (1999).
4. Ferry, G., et al., Development of new assays and improved procedures for the purification of recombinant human chymase. *Eur. J. Biochem.*, **268**, 5885-5893 (2001).
5. Tani, M., et al., SF2809 Compounds, novel chymase inhibitors from *Dactylosporangium* sp. 1. Taxonomy, fermentation, isolation and biological properties. *J. Antibiotics*, **57**, 83-88 (2004).
6. Greco, M.N., et al., Discovery of potent, selective, orally active, nonpeptide inhibitors of human mast cell chymase. *J. Med. Chem.*, **50**, 1727-1730 (2007).

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