Product Information

3050 Spruce Street, Saint Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Carboxypeptidase A Assay Kit

Catalog Number **CS1130**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Carboxypeptidase A (CPA) catalyzes the hydrolysis of C-terminal aromatic or aliphatic amino acids of proteins or peptides. Carboxypeptidase A is found in the pancreas and in mast cells. Carboxypeptidase A found in mast cells (MC-CPA) is a highly conserved secretory granule protease that is important for mast cell differentiation.[1] MC-CPA levels rise during anaphylaxis, a severe acute allergic reaction.[2] This enzyme is considered to be a sensitive marker for anaphylaxis, much more sensitive than the present marker tryptase. It has been suggested that MC-CPA can degrade snake venom components and honeybee venoms, thus, having a protective function during envenomation.[3]

The kit allows the fast and convenient determination of CPA activity in biological samples, as well as screening for enzyme inhibitors. The kit contains all the necessary components including a Carboxypeptidase A control enzyme and Carboxypeptidase Inhibitor.

The CPA activity assay is based on the hydrolysis of the substrate N-(4-methoxyphenylazoformyl)-Phe-OH. The substrate has a strong absorption peak at 350 nm (ε^{mM} = 19.0). The reaction progression is reflected by a decrease in absorption at 350 nm.[4]

Unit definition: 1 unit will hydrolyze 1 μmole of N-(4-methoxyphenylazoformyl)-Phe-OH per minute at pH 8 at 25 °C.

Components

The kit is sufficient for 200 assays in 96 well plates or 40 assays in 1 mL cuvette.

Assay Buffer Catalog Number A7731	5 mL
Substrate Solution Catalog Number S8697	50 μL
Carboxypeptidase A from bovine pancreas Catalog Number C8368	200 μL
Carboxypeptidase Inhibitor from potato tuber Catalog Number C8493	200 μL
Sodium Carbonate Catalog Number S2127	2.5 g

Reagents and Equipment Required but Not Provided

- 96 well clear flat bottom plate (Catalog Number CLS3358)
- Plate reader or spectrophotometer
- 1 mL quartz cuvette (optional)
- Ultrapure water
- Magnetic stirrer and stir bar

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

Use ultrapure water (17 MΩ·cm or equivalent) for the preparation of reagents and throughout the procedure.

Stop Solution - Add 24 mL of ultrapure water to the sodium carbonate bottle (Catalog Number S2127) and mix well on a magnetic stirrer until completely dissolved. Store the Stop Solution at room temperature.

Reaction Mixture – Prepare 1 mL of reaction mixture by mixing 800 μL of ultrapure water, 200 μL of Assay Buffer (Catalog Number A7731), and 2 μL of the Substrate Solution (Catalog Number S8697). One mL of Reaction Mixture is sufficient for 2 assays in 1 mL cuvette or 10 assays in a 96 well plate.

Storage/Stability

The kit is shipped on dry ice and storage at –20 °C is recommended. Upon arrival store the Sodium Carbonate (Catalog Number S2127) at room temperature.

Procedure
The following procedure is for 96 well plate assays. Perform the reactions in duplicates.

Notes: The 96 well plate assay is based on a fast end-point reaction. Therefore, if several reactions are performed simultaneously, it is highly recommended to use a multichannel pipette for initiation and termination of the reaction.

It is possible to perform the assay in cuvettes. The volumes of the reagents should be adjusted according to the increase in the reaction volume.

Table 1.
Reaction scheme for 96 well plate assay

Sample	Enzyme	Inhibitor	Water	Reaction Mixture
Blank	–	–	100 µL	100 µL
Test sample	x µL sample	–	100–x µL	100 µL
Positive Control	1–2 µL Carboxy-peptidase A	–	97–98 µL	100 µL
Control with Inhibitor	2 µL Carboxy-peptidase A	1 µL	97 µL	100 µL

x - volume of the test sample added to the reaction

1. Prepare a blank control by adding 100 µL of ultrapure water to the appropriate wells.
2. Prepare a test sample reaction by adding 1–50 µL (1–100 µg protein) of sample to the appropriate wells. Adjust the volume to 100 µL with ultrapure water.
3. Prepare a positive control by adding 1–2 µL of the Carboxypeptidase A control enzyme to the appropriate wells. Adjust the volume to 100 µL with ultrapure water.
4. Prepare a control with inhibitor reaction by adding 2 µL of the Carboxypeptidase A control enzyme and 1 µL of Carboxypeptidase Inhibitor to the appropriate wells. Adjust the total volume to 100 µL with ultrapure water.
   Note: If other enzyme preparations or/and inhibitors are used, their volumes must be optimized based on their activities. Add to a well the appropriate amounts of inhibitor and enzyme of choice, and adjust the total volume to 100 µL with ultrapure water. Then continue with the procedure.
5. Start the reaction by the addition of 100 µL of Reaction Mixture to each well.
6. Incubate the plate for 3–5 minutes at 25 °C.
7. Stop the reaction with 100 µL of Stop Solution.
   Note: Some detergents (e.g., TRITON® X-100) may precipitate when the sodium carbonate Stop Solution is added. If a precipitate forms, measure the absorption without the addition of Stop Solution.
8. Read the absorption at 350 nm.

Results
Calculation
The reaction rate is linear only when the decrease in absorption (A_{350}) does not exceed 0.35. For calculating the CPA activity according to the following equations, use only the linear range of the reaction.

Calculation of the CPA activity detected using 96 well plate:

$$\text{CPA Activity (milliunits/mL)} = \frac{\Delta A_{350} \times \text{Dilution} \times 0.2}{\text{Time} \times 0.019 \times 0.55 \times V_{sample}}$$

Calculation of the CPA activity detected using a 1 mL cuvette:

$$\text{CPA Activity (milliunits/mL)} = \frac{\Delta A_{350} \times \text{Dilution}}{\text{Time} \times 0.019 \times V_{sample}}$$

Where:
ΔA_{350} - difference between initial and final absorption at 350 nm
Note: initial absorption is equal to the blank control absorption
Dilution - dilution of the test sample or enzyme stock
Time - reaction duration (incubation time) from the beginning of the reaction to the end (minutes)
V_{sample} - volume of the sample/control enzyme added to the reaction mixture (mL)
0.019 - µmolar extinction coefficient of the substrate
0.2 - volume of reaction in mL
0.55 - effective pathlength in 96 well plates

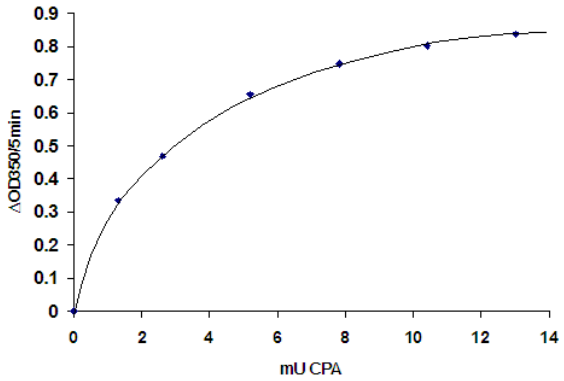
Calibration curve

The calibration curve for the supplied Carboxypeptidase A control is not linear (see Figure 1). Therefore, to determine the activity of an unknown sample, which is not in the linear range of the reaction, a calibration curve should be used. The activity of the unknown sample may be determined by this curve.

Determination of a Carboxypeptidase A calibration curve:

1. Dilute 40 µL of the Carboxypeptidase A control enzyme (Catalog Number C8368) 10-fold with ultrapure water.
2. Place 0, 10, 20, 40, 60, and 80 µL of the diluted Carboxypeptidase A control enzyme into the appropriate wells. Bring the volume to 100 µL with ultrapure water.
3. Start the reaction by the addition of 100 µL of Reaction Mixture.
4. Incubate the plate for 5 minutes at 25 °C.
5. Stop the reaction with 100 µL of Stop Solution.
6. Read the absorbance at 350 nm.
7. Plot the calibration curve (ΔOD_{350} per minute as a function of CPA activity, see Figure 1).
   Note: CPA activity of the control enzyme must be calculated according to the equation in the Results section, using a single point where the reaction rate is still in the linear range: Use the ΔOD_{350} (ΔA_{350}) value for the first point (10 µL) of the control enzyme in the equation and determine the activity in milliunits. Calculate the activity for the rest of the enzyme calibration curve samples according to the value obtained for the 10 µL sample (e.g., if there are 0.9 milliunits in the 10 µL sample, then the 20 µL sample contains 1.8 milliunits).

Figure 1.
Carboxypeptidase A Calibration Curve



Calibration Curve for the control Carboxypeptidase A provided with this kit. It is possible that a slightly different curve may be obtained for CPA from other biological sources.

References

1. Feyerabend, T.B., et al., Loss of histochemical identity in mast cells lacking Carboxypeptidase A. *Mol. Cell Biol.,* **25**, 6199–6210 (2005).
2. Zhou, X., et al., Mast cell Carboxypeptidase as a new clinical marker for anaphylaxis. *J. Allergy Clin. Immunol*., **117**, S85 (2006).
3. Metz, M., et al., Mast cells can enhance resistance to snake and honeybee venoms. *Science*, **313**, 526-530 (2006).
4. Mock, W.L., et al., Arazoformyl peptide surrogates as spectrophotometric kinetic assay substrates for Carboxypeptidase A. *Anal. Biochem*., **239**, 218-222 (1996).

EB, MAM 01/08-1