Version 4a, Last updated 13 June 2025

ab239722 Pepsin/Pepsinogen Assay Kit (Fluorometric)

For the measurement of pepsin in biological samples.

This product is for research use only and is not intended for diagnostic use.

Table of Contents

1. Overview

Pepsin/Pepsinogen Assay Kit (Fluorometric) (ab238722) is a homogenous assay that allows for quantification of pepsin activity in gastric tissues (stomach, duodenum etc.) and various biological fluids (serum/plasma, gastric juice, vomit). The assay utilizes a synthetic peptide substrate bearing both a fluorophore and a fluorescence quencher. Upon cleavage by pepsin, the fluorophore-bearing peptide fragment is unquenched to produce a bright fluorescent signal (Ex/Em = 328/418 nm). Lysosomal aspartic proteases in the peptidase A1 family (Cathepsin D and E) do not interfere with the assay. The assay is rapid, simple to perform and is vastly more sensitive than the classical hemoglobin degradation assay, with a detection limit of 500 µU pepsin activity per well.

Prepare tissue / serum / plasma sample and Pepsin Positive Control.

⇩

Prepare Standard Curve.

⇩

Prepare Reaction Mix, Inhibitor Reaction Mix, Background Control Mix and Positive Control Reaction Mix.

⇩

Start reaction by adding 20 µL diluted Substrate working solution to each well (apart from Standard Curve).

⇩

Measure the fluorescence (Ex/Em = 328/418 nm) of all sample wells in kinetic mode for 60 minutes at 37°C.

2. Materials Supplied and Storage

Store kit at -20°C in the dark immediately on receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.

Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
Pepsin Assay Buffer	25 mL	-20°C	-20°C
Substrate II	0.2 mL	-20°C	-20°C
Pepstatin A Solution	20 µL	-20°C	-20°C
Pepsin Positive Control	1 vial	-20°C	-20°C
MCA Standard	25 µL	-20°C	-20°C

3. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate reader capable of measuring fluorescence at Ex/Em = 328/418 nm.
- White 96-well plate with clear flat bottom.

4. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:
www.abcam.com/assaykitguidelines

For typical data produced using the assay, please see the assay kit datasheet on our website.

5. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

5.1 Substrate II

Provided as a stock solution in DMSO. Divide into aliquots and store at -20°C, protected from light. Prior to use, warm solution to room temperature and vortex thoroughly.

5.2 Pepstatin A Solution

Provided as a 1 mM stock solution in DMSO. Prior to use, warm solution to room temperature and vortex thoroughly. Store at -20°C, stable for at least 3 freeze/thaw cycles.

5.3 Pepsin Positive Control

Reconstitute with 110 µL ddH2O. Divide into aliquots and store at -20°C. Avoid repeated freeze/thaw cycles

5.4 MCA Standard

Provided as a 1 mM stock solution in DMSO. Warm solution to room temperature prior to use. Store at -20°C, protected from light. Stable for at least 3 freeze/thaw cycles.

5.5 Pepsin Assay Buffer

Ready to use as supplied. Warm to room temperature prior to use.

6. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

6.1 Prepare a 50 µM working solution of MCA by adding 5 µL of the 1 mM MCA Standard to 95 µl of Pepsin Assay Buffer.

6.2 Add 0, 2, 4, 6, 8, and 10 µL of the 50 µM working solution into a series of wells in a white 96-well plate, generating 0, 100, 200, 300, 400 and 500 pmol of MCA/well. Adjust the volume of all standard curve wells (including the 0 pmol/well reagent blank) to 100 µL with Pepsin Assay Buffer.

Standard #	MCA Standard (µL)	Pepsin Assay Buffer (µL)	Final volume standard in well (µL)	MCA Standard (pmol/well)
1	20	180	200	500
2	16	184	200	400
3	12	188	200	300
4	8	192	200	200
5	4	196	200	100
6	0	200	200	0

Each dilution has enough standard to set up duplicate readings (2 x 100 µL).

7. Sample Preparation

General sample information:

We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.

We recommend that you use fresh samples for the most reproducible assay.

7.1 Mammalian tissue samples:

1. Homogenize fresh or frozen tissue with Pepsin Assay Buffer (100 µL per ~10 mg of wet tissue) on ice and vortex thoroughly. Centrifuge the homogenate at 4°C for 10 minutes at 10,000 x *g.*
2. Transfer the clarified supernatant to a fresh pre-chilled microfuge tube and keep on ice during use. Add 5-20 µL of clarified homogenate to desired well(s) in a white, flat-bottomed 96-well plate.

7.2 Serum and plasma samples:

1. Collect serum or plasma samples by standard methods. Samples exhibiting lipemia or excessive turbidity should be clarified by centrifugation at 10,000 x *g* for 5 minutes in order to separate lipid globules.
2. Add 5-20 µL of undiluted serum/plasma sample to desired well(s).

7.3 Pepsin positive control:

1. Dilute the reconstituted Pepsin Positive Control at 1:10 ratio by mixing 10 µL of the reconstituted stock with 90 µL of Pepsin Assay Buffer.
2. Incubate the diluted Pepsin Positive Control solution at room temperature for 5 minutes to allow for acid-mediated pepsin auto-activation, then add 10 µL of diluted Pepsin Positive Control solution to desired well(s).

ΔNote: Pepsin undergoes autolysis (self-cleavage) in solution at pH values ≤ 4. To prevent pepsin autolysis, we recommend storing tissue homogenate samples at -80°C if they are to be used in future experiments.

8. Assay Procedure

– Assay all standards, controls and samples in duplicate.

8.1 Prepare assay reaction wells according to the table below. In addition to the test sample wells, prepare a background control (substrate only) well to correct for potential non-enzymatic substrate hydrolysis.

8.2 For further verification of pepsin activity, you may prepare inhibitor control wells (sample + 1 µM Pepstatin A). To prepare inhibitor control wells, dilute the Pepstatin A Solution stock solution at 1:100 ratio in Pepsin Assay Buffer to produce a 10 µM working solution and add 10 µL of the working solution per well.

Component	Reaction Mix (µL)	Plus Inhibitor Mix (µL)	Background Control Mix (µL)	Positive Control (µL)
Test sample	5-20	5-20	-	-
Diluted Pepsin Positive control	-	-	-	10
Pepstatin A	-	10	-	-
Pepsin Assay Buffer	to 80 µL	to 80 µL	80	70

8.3 Preincubate the plate for 10 min at 37°C to allow sample temperature to equilibrate. During the preincubation, prepare Substrate II working solution by diluting the Substrate II stock solution with Pepsin Assay Buffer at a 1:10 ratio. Prepare 20 µL of substrate working solution for each reaction to be performed (for example, for 10 reaction wells, mix 20 µL of Substrate II stock with 180 µL Pepsin Assay Buffer).

8.4 Start the reaction by adding 20 µL of the diluted substrate working solution to each reaction well, yielding a final volume of 100 µL per well.

ΔNote: Do not add Substrate II solution to the MCA Standard Curve wells.

8.5 Measure the fluorescence (Ex/Em = 328/418 nm) of all sample wells in kinetic mode for 60 min at 37°C. We strongly recommend reading in kinetic mode in order to ensure that

the measurements recorded are within the linear range of the reaction. Ideal measurement time for the linear range may vary depending upon the sample.

ΔNote: The MCA Standard curve wells may be read in endpoint mode (Ex/Em = 328/418 nm).

9. Data Analysis

Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiply the concentration found by the appropriate dilution factor.

1. Average the duplicate reading for each standard, control and sample.
2. For the MCA Standard curve, subtract the fluorescence obtained for the reagent blank (0 pmol/well standard) from all of the standard readings, plot the background-subtracted values and calculate the slope of the standard curve.
3. For all reaction wells (including background control), choose two time points (t_1 and t_2) in the linear phase of the reaction progress curves, obtain the corresponding fluorescence values at those points (RFU_1 and RFU_2) and determine the change in fluorescence over the time interval: $\Delta F = RFU_2 – RFU_1$.
4. If the ΔF value for the background control well is significant, it should be subtracted from each test sample to obtain the corrected fluorescence: $FC = \Delta F_{sample} – \Delta F_{BC}$. If ΔF_{BC} is negative, background subtraction should be ignored: $FC = \Delta F_{sample}$.
5. Apply the FC values to the MCA Standard curve to get B pmol of unquenched MCA-peptide in the well.

$$\text{Sample Pepsin Activity} = \frac{B}{\Delta T * P} * D \quad \textit{= pmol/min/(mL or mg)}$$

Where:

B = amount of peptide substrate cleaved, calculated from the Standard Curve (in pmol).

ΔT = linear phase reaction time $t_1 – t_2$ (in minutes).

P = the amount of sample added to the well (in ml of biological fluid or mg of protein)

D = sample dilution factor applied (if applicable, D = 1 for undiluted samples).

Pepsin Unit Definition: One unit of pepsin activity is the amount of enzyme that generates 1 µmole of unquenched 7-methoxycoumarin-4-acetate (MCA) per min by hydrolysis of 1 µmole peptide substrate at 37°C and pH 2.

10. Typical Data

Data provided **for demonstration purposes only.**

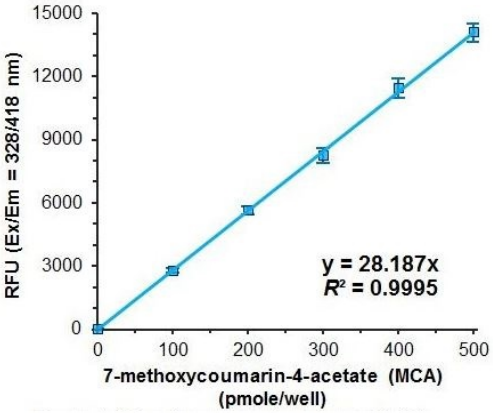

Figure 1. MCA standard curve.

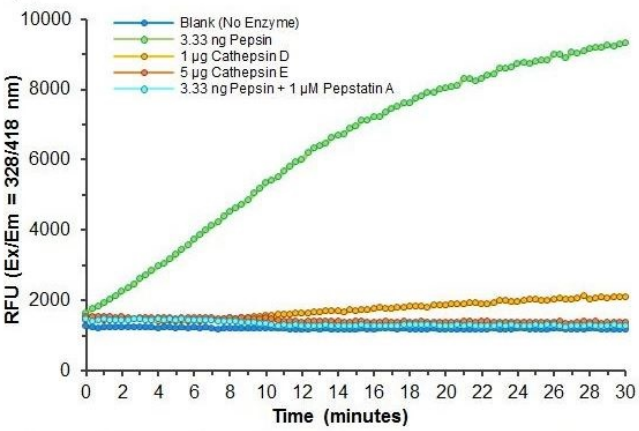

Figure 2. Kinetics of Substrate II metabolism by pig gastric mucosal pepsin (3.33 ng purified enzyme) and specificity of substrate metabolism by pepsin versus other aspartic proteases. The acid-activated proteases Cathepsin D

and E exhibit minimal assay interference, even when present at ≥300-fold excess by mass.

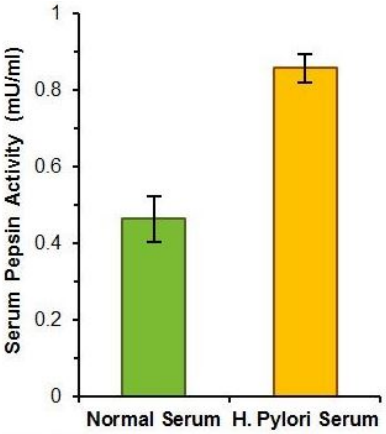

Figure 3. Estimation of pepsinogen activity in pooled normal human serum and single-donor serum from a gastric ulcer patient with confirmed H. Pylori infection (each 10 µL of undiluted serum). Data are mean ± SEM of 3 replicates.

11. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)