ab126780

Cathepsin G Activity Assay Kit

Instructions for Use

For the rapid, sensitive and accurate measurement of Cathepsin G activity in various samples

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

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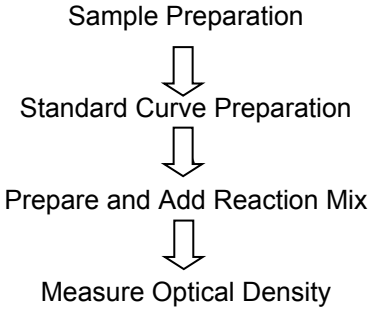
1. Overview

Cathepsin G is an enzymatic protein belonging to the peptidase or protease families. The protein is found in azurophil granules of neutrophilic polymorphonuclear leukocytes. The encoded protease has a specificity similar to that of chymotrypsin C, and may participate in the killing and digestion of engulfed pathogens, and in connective tissue remodeling at sites of inflammation.

In Abcam's Cathepasin G Activity assay Kit, Cathepsin G will cleave the substrate and release the dye group, *p*-NA (4-Nitroaniline), which can be detect at 405 nm. In presence of the Cathepsin G specific inhibitor, the cleavage will be stopped. The kit provides a rapid, simple, sensitive, and reliable test suitable for the activity of Cathepsin G.

2. Protocol Summary



3. Components and Storage

A. Kit Components

Item	Quantity
Assay Buffer 66	25 mL
Cathepsin G Substrate	200 µL
Cathepsin G Inhibitor	20 µL
pNA Standard I	20 µL
Human Cathepsin G	1 vial

PLEASE NOTE: Assay Buffer 66 was previously labelled as Assay Buffer XLVI and Assay Buffer, and pNA Standard I as p-NA Standard (0.1 M), and [Human Cathepsin G] as [Positive Control (Lyophilized)]. The composition has not changed.

* Store kit at -20°C, protect from light.

- Warm the assay buffer to room temperature before use.
- Briefly centrifuge vials before opening.
- Read the entire protocol before performing the assay.

HUMAN CATHEPSIN G: Reconstitute with 20 µl dH_2O. Store in -20°C, avoid thaw and freeze cycle, use within 1 month.

B. **Additional Materials Required**

- Microcentrifuge
- Pipettes and pipette tips
- Fluorescent microplate reader
- 96-well plate
- Orbital shaker

4. Assay Protocol

1. Sample Preparation:

For cell samples: Collect cells (10^6) by centrifugation.. Lyse cells in 50 µl of chilled Assay Buffer 66. Incubate cells on ice for 10 minutes. Centrifuge 13,000 rpm for 5 min in bench-top micro-centrifuge to remove insoluble materials. Transfer the clear lysate into a new tube. Measure protein concentration if desired.

Prepare duplicate **test samples** up to 50 µl/well with Assay Buffer 66 in a 96-well plate.

For **background control** dilute Cathepsin G Inhibitor 1:50 with Assay Buffer 66. Add 10 µl Assay Buffer 66 to one test sample and 10 µl diluted Cathepsin G Inhibitor to the duplicated sample as the sample background control. Mix well and incubate for 10 min. at 37°C.

For **positive control** (optional) use 2-5 µl Human Cathepsin G Solution and adjust volume to 50 µl.

We suggest testing several doses of your sample to make sure readings are within the standard curve.

2. Standard Curve Preparation:

Dilute 5 µl 0.1 M *p*-NA (4-Nitroaniline) standard solution into 95 µl assay buffer to prepare 5 mM *p*-NA.

Add 0, 2, 4, 6, 8, 10 µl 5 mM pNA Standard I into each well individually. Adjust volume to 100 µl/well with Assay Buffer 66 to generate 0, 10, 20, 30, 40, 50 nmol/well of pNA Standard I. Read OD at 405 nm.

3. Substrate Solution: Mix enough reagents for the number of assays to be performed. For each well, prepare a total 40 µl Substrate Solution:

Assay Buffer 66	38 µl
Substrate	2 µl

Add 40 µl Substrate Solution into each sample well. Mix well. **(Do Not Add to Standard Curve Wells)**

4. Measurement: Read OD at 405 nm A_{S1} and A_{B1} at T_1. Read A_{S2} and A_{B2} again at T_2 after incubating the reaction at 37°C for 60 min, protected from light. The OD generated by hydrolyzation of substrate by Cathepsin G is

$$\mathbf{\Delta A = (A_{S2} - A_{S1}) - (AB_2 - AB_1).}$$

Note:

It is recommended to read kinetically to choose the A_{S1} and A_{S2} in the linear range and which falls within the Standard Curve.

5. Data Analysis

Plot pNA Standard Curve, Apply the ΔA to the Standard Curve to get B nmol of *p*-NA:

$$\textbf{Cathepsin G Activity} = \frac{\textbf{(B x Dilution Factor)}}{\textbf{(}T_2 - T_1\textbf{) x V}} = \textbf{nmol/min/ml = mU/ml}$$

Where:

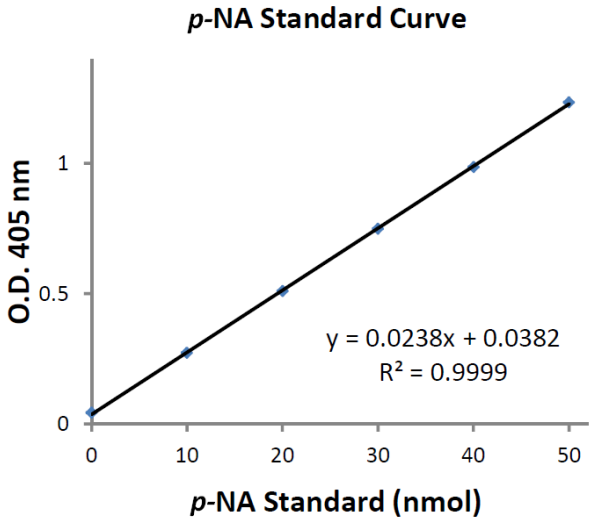
B is the *p*-NA amount (nmol) from the Standard Curve

$\mathbf{T_1}$ is the time (min) of the first reading (A_{S1} and A_{B1})

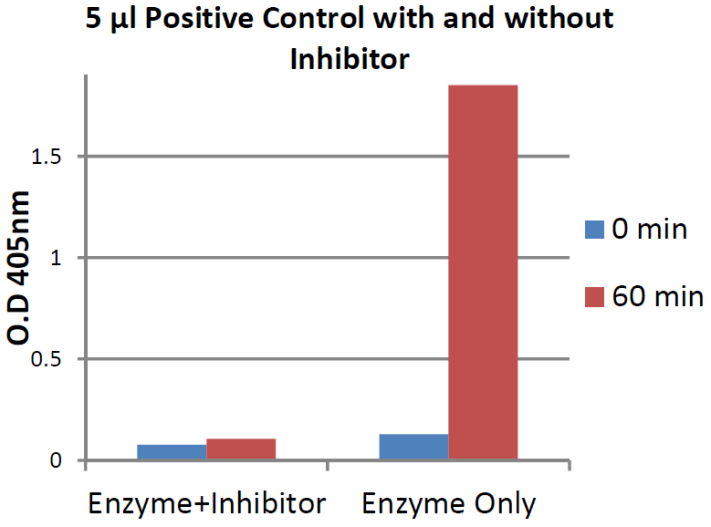
$\mathbf{T_2}$ is the time (min) of the second reading (A_{S2} and A_{B2})

V is the sample volume (ml) added into the reaction well

Unit Definition: One unit is defined as the amount of Cathepsin G that hydrolyzes the substrate to yield 1.0 µmol of *p*-NA per minute at 37°C.



pNA Standard Curve: Performed according to assay procedure.



6. Troubleshooting

Problem	Reason	Solution
Assay not working	Assay buffer at wrong temperature	Assay buffer must not be chilled - needs to be at RT
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range

Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Use the assay buffer provided (or refer to datasheet for instructions)
	Samples not deproteinized (if indicated on datasheet)	Use the ***10kDa spin column (ab93349)***
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freeze-thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
Lower/ Higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/ temperature	Refer to datasheet for recommended incubation time and/ or temperature
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)

Problem	Reason	Solution
Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes
	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Recheck datasheet for recommended concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit

For further technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select "*contact us*" on www.abcam.com for the phone number for your region).

Technical Support

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