PRODUCT INFORMATION & MANUAL

Cathepsin G Activity Assay Kit (Colorimetric)

NBP2-54849

For research use only.
Not for diagnostic or therapeutic procedures.

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Cathepsin G Activity Colorimetric Assay Kit

(Catalog #NBP2-54849 Store kit at -20°C)

I. Introduction: 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 Novus'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.

II. Kit Contents:

Components	100 assays	Cap Color
Assay Buffer	25 ml	WM
Substrate	200 µl	Red
Cathepsin G Inhibitor	20 µl	Blue
*p*NA Standard (0.1 M)	20 µl	Yellow
Positive Control	1 vial	Green

III. Storage and Handling:

Store the kit at -20°C, protect from light. Allow Assay Buffer to warm to room temperature before use. Briefly centrifuge vials before opening. Read the entire protocol before performing the assay.

Reconstitute positive control with 20 µl dH2O. Store in -20°C, avoid thaw and freeze cycle, good for using in 1 month.

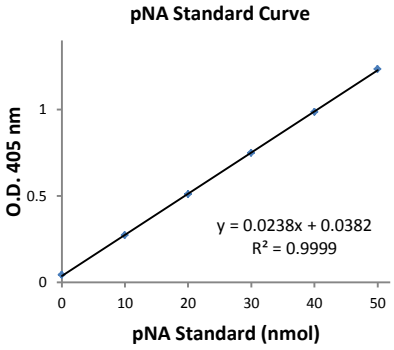
IV. Cathepsin Activity assay Protocol:

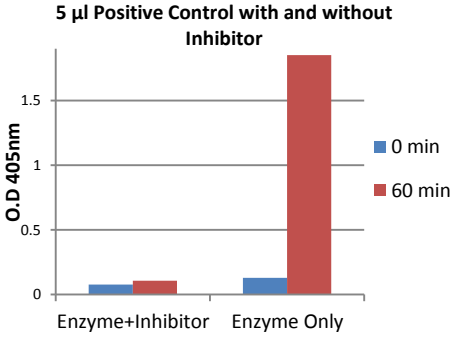
1. **Standard Curve Preparation:**
   Dilute 5 µl 0.1 M *p*NA (4-Nitroaniline) standard into 95 µl assay buffer to prepare 5 mM *p*NA. Add 0, 2, 4, 6, 8, 10 µl 5 mM *p*NA standard into each well individually. Adjust volume to 100 µl/well with Assay Buffer to generate 0, 10, 20, 30, 40, 50 nmol/well of *p*NA standard. Read O.D at 405 nm.
2. **Sample Preparations:**
   Collect cells (10^6) by centrifugation.. Lyse cells in 50 µl of chilled Assay Buffer. Incubate cells on ice for 10 minutes. Centrifuge 13000 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. Serum samples can be directly diluted in the Assay Buffer. Prepare duplicate test samples of up to 50 µl/well with Assay Buffer in a 96-well plate. We suggest testing several doses of your sample to make sure the readings are within the standard curve range. For optional Cathepsin G Positive Control use 2-5 µl and adjust volume to 50 µl.
3. **Set Background Control**
   Dilute Cathepsin G Inhibitor 1:50 with Assay Buffer. Add 10 µl Assay Buffer 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.
4. **Substrate Solution Preparation:**
   38 µl Assay Buffer
   2 µl Substrate
   Add 40 µl Substrate Solution into each sample well (Do Not Add to Standard Curve Wells). Mix well.
5. **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 $\Delta A = (A_{S2} - A_{S1}) - (A_{B2} - A_{B1})$. 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.
6. **Calculation:** Plot *p*NA Standard Curve, Apply the ΔA to the Standard Curve to get B nmol of *p*NA:

$$\text{Activity} = \frac{B}{(T2-T1)\times V} \times \text{Sample Dilution Factor} = \text{nmol/min/ml} = \text{mU/ml}$$

Where: B is the *p*NA amount from Standard Curve (in nmol).
T_1 is the time of the first reading (A_{s1} and A_{B1}) (in min).
T_2 is the time of the second reading (A_{S2} and A_{B2}) (in min).
V is the sample volume added into the reaction well (in ml).

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.





GENERAL TROUBLESHOOTING GUIDE

Problems	Cause	Solution
Assay not working	• Cells did not lyse completely	• Resuspend the cell pellet in the lysis buffer and incubate as described in the datasheet
	• Experiment was not performed at optimal time after apoptosis induction	• Perform a time-course induction experiment for apoptosis
	• Plate read at incorrect wavelength	• Check the wavelength listed in the datasheet and the filter settings of the instrument
High Background	• Increased amount of cell lysate used	• Refer to datasheet and use the suggested cell number to prepare lysates
	• Increased amounts of components added due to incorrect pipetting	• Use calibrated pipettes
	• Incubation of cell samples for extended periods	• Refer to datasheet and incubate for exact times
	• Use of expired kit or improperly stored reagents	• Always check the expiry date and store the individual components appropriately
	• Contaminated cells	• Check for bacteria/ yeast/ mycoplasma contamination
Lower signal levels	• Cells did not initiate apoptosis	• Determine the time-point for initiation of apoptosis after induction (time-course experiment)
	• Very few cells used for analysis	• Refer to datasheet for appropriate cell number
	• Use of samples stored for a long time	• Use fresh samples or aliquot and store and use within one month for the assay
	• Incorrect setting of the equipment used to read samples	• Refer to datasheet and use the recommended filter setting
	• Allowing the reagents to sit for extended times on ice	• Always thaw and prepare fresh reaction mix before use
Samples with erratic readings	• Uneven number of cells seeded in the wells	• Seed only equal number of healthy cells (correct passage number)
	• Samples prepared in a different buffer	• Use the cell lysis buffer provided in the kit
	• Adherent cells dislodged and lost at the time of experiment	• Perform experiment gently and in duplicates/triplicates; apoptotic cells may become floaters
	• Cell/ tissue samples were not completely homogenized	• Use Dounce homogenizer (increase the number of strokes); observe efficiency of lysis under microscope
	• Samples used after multiple freeze-thaw cycles	• Aliquot and freeze samples, if needed to use multiple times
	• Presence of interfering substance in the sample	• Troubleshoot as needed
	• Use of old or inappropriately stored samples	• Use fresh samples or store at correct temperatures until use
Unanticipated results	• Measured at incorrect wavelength	• Check the equipment and the filter setting
	• Cell samples contain interfering substances	• Troubleshoot if it interferes with the kit (run proper controls)
General issues	• Improperly thawed components	• Thaw all components completely and mix gently before use
	• Incorrect incubation times or temperatures	• Refer to datasheet & verify the correct incubation times and temperatures
	• Incorrect volumes used	• Use calibrated pipettes and aliquot correctly
	• Air bubbles formed in the well/tube	• Pipette gently against the wall of the well/tubes
	• Substituting reagents from older kits/ lots	• Use fresh components from the same kit
	• Use of a different 96-well plate	• Fluorescence: Black plates; Absorbance: Clear plates

Note# The most probable cause is listed under each section. Causes may overlap with other sections.