ab204693
Cathepsin G Inhibitor Screening Assay Kit (Colorimetric)

Instructions for Use

For rapid, sensitive and accurate screening of potential Cathepsin G inhibitors.

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

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

Version 3b, Last Updated 5 October 2023

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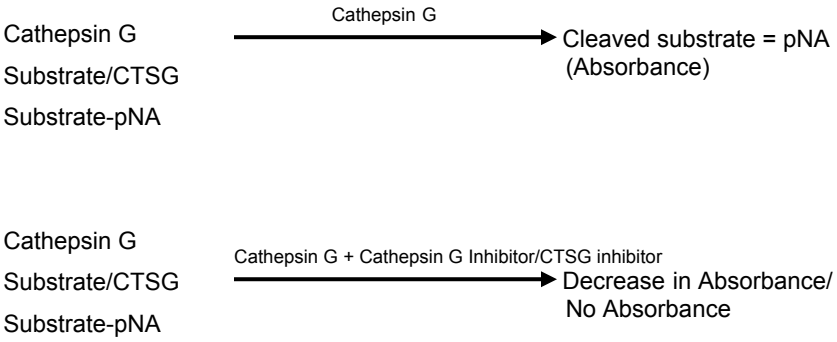
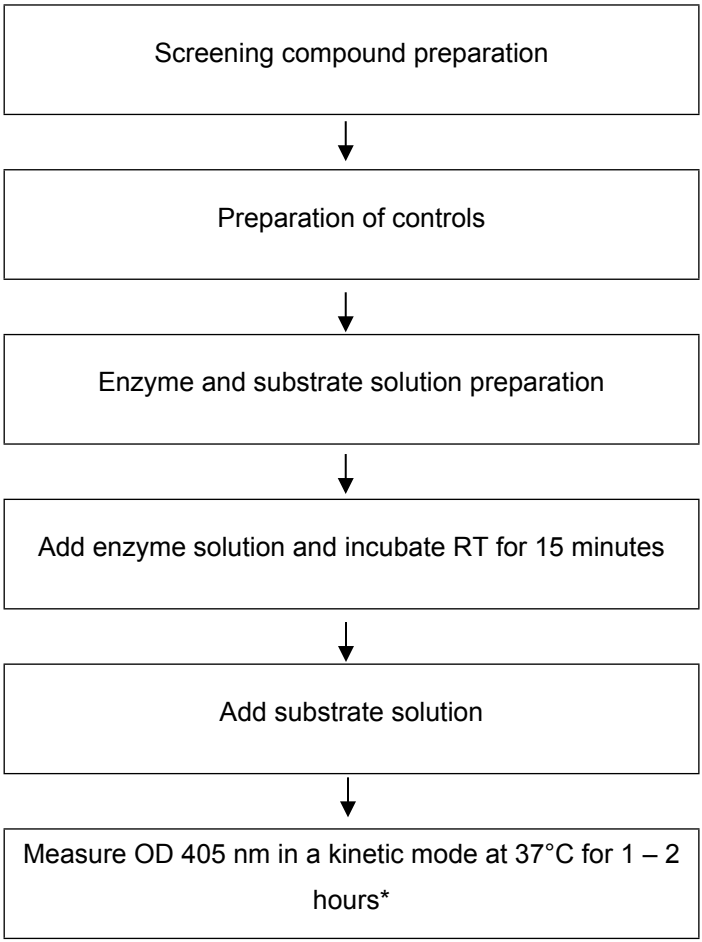
1. BACKGROUND

Cathepsin G Inhibitor Screening Assay Kit (colorimetric) (ab204693) uses the ability of active Cathepsin G to cleave a synthetic pNA (4-Nitroaniline)-based peptide substrate to release pNA, which can be easily quantified using a colorimetric microplate reader. In the presence of a Cathepsin G inhibitor, the cleavage of this substrate is reduced/abolished resulting in decrease or total loss of the pNA absorbance. This simple and high-throughput adaptable assay kit can be used to screen, study or characterize potential inhibitors of Cathepsin G.



Cathepsin G (CTSG, EC 3.4.21.20) is a serine protease found in azurophil granules of neutrophilic polymorphonuclear leukocytes. The encoded protease has specificity similar to that of chymotrypsin C, and may participate in the killing and digestion of engulfed pathogens, and in connective tissue remodelling at sites of inflammation.

2. ASSAY SUMMARY



**For kinetic mode detection, incubation time given in this summary is for guidance only.*

3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at -20ºC in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature. **Reconstituted components are stable for 2 months.**

5. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not use kit or components if it has exceeded the expiration date on the kit labels.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)	Storage Condition (After Preparation)
Cathepsin G Assay Buffer/CTSG Assay Buffer	20 mL	-20°C	-20°C
Human Cathepsin G II/Cathepsin G (human)	1 vial	-20°C	-80°C
Cathepsin G Substrate/CTSG Substrate	200 µL	-20°C	-20°C
Cathepsin G Inhibitor/CTSG Inhibitor (0.5 mM)	20 µL	-20°C	-20°C

7. MATERIALS REQUIRED, NOT SUPPLIED

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

- Inhibitor compound of choice
- Pipettes and pipette tips
- Microcentrifuge
- Colorimetric microplate reader – equipped with filer for OD = 405 nm
- 96 well plate: clear plate with flat bottom
- Heat block or water bath

8. TECHNICAL HINTS

- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required.
- Keep enzymes and heat labile components and samples on ice during the assay.
- Make sure all buffers and developing solutions are at room temperature before starting the experiment.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Ensure plates are properly sealed or covered during incubation steps.
- Make sure you have the appropriate type of plate for the detection method of choice.
- Make sure the heat block/water bath and microplate reader are switched on before starting the experiment.

9. REAGENT PREPARATION

- Briefly centrifuge small vials at low speed prior to opening.

9.1 **Cathepsin G Assay Buffer/CTSG Assay Buffer:**

Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

9.2 **Human Cathepsin G II/Cathepsin G (human):**

Reconstitute in 110 µL of Cathepsin G Assay Buffer/CTSG Assay Buffer. Mix by gently pipetting up and down. Aliquot so that you have enough volume to perform the desired number of assays. Store at -80°C. Avoid repeated freeze/thaw.

9.3 **Cathepsin G Substrate/CTSG Substrate:**

Ready to use as supplied. Aliquot so that you have enough volume to perform the desired number of assays. Store at -20°C.

9.4 **Cathepsin G Inhibitor/CTSG Inhibitor (0.5 mM):**

Ready to use as supplied. Aliquot so that you have enough volume to perform the desired number of assays. Store at -20°C.

10. SAMPLE PREPARATION

- Always prepare a fresh set of samples and controls for every use.

10.1 Screening Compounds:

10.1.1 Dissolve test compounds into appropriate solvent.

10.1.2 Dilute to 10X the desired test concentration with Cathepsin G Assay Buffer/CTSG Assay Buffer before use.

NOTE: *We suggest using different volumes of testing compounds if effective concentration is unknown.*

11. ASSAY PROCEDURE and DETECTION

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **It is recommended to assay all controls and samples in duplicate.**

11.1 **Set up reaction wells:**

- Sample wells (S) = 10 µL test inhibitors.
- Inhibitor Control wells (IC) = 1 µL Cathepsin G Inhibitor/CTSG inhibitor (0.5 mM) + 9 µL Cathepsin G Assay Buffer/CTSG Assay Buffer.
- Enzyme Control wells (EC) = 10 µL Cathepsin G Assay Buffer/CTSG Assay Buffer.
- OPTIONAL: Solvent control (SC) = 10 µL solvent.
  NOTE: *preferred final solvent concentration should not be more than 2% by volume. If solvent exceeds 2%, include solvent control to test the effect on the solvent on enzyme activity.*

11.2 **Prepare Cathepsin G Enzyme Solution:**

Prepare 50 µL of Cathepsin G Enzyme Solution for each well:

Component	Enzyme Solution (µL)
Cathepsin G Assay Buffer/CTSG Assay Buffer	**49**
Human Cathepsin G II/Cathepsin G Enzyme	**1**

Mix enough reagents for the number of assays (samples and controls) to be performed. Prepare a master mix of the Reaction Mix to ensure consistency. We recommend the following calculation: X µL component x (Number reactions +1)

11.3 Add 50 µL of CTSG Enzyme Solution to each well. Mix gently.

11.4 Incubate at room temperature for 10-15 minutes.

11.5 **Cathepsin G Substrate/CTSG Substrate Solution:**

Prepare 40 µL of GTSG Substrate Solution for each well:

Component	Substrate Solution (µL)
Cathepsin G Assay Buffer/CTSG Assay Buffer	38
Cathepsin G Substrate/CTSG Substrate	2

Mix enough reagents for the number of assays (samples and controls) to be performed. Prepare a master mix of the Reaction Mix to ensure consistency. We recommend the following calculation: X µL component x (Number reactions +1)

Table below shows the experimental set up:

Component	Sample Well (S) (µL)	Inhibitor Control (IC) (µL)	Enzyme control (EC) (µL)
Test inhibitor compound	10	0	0
Cathepsin G Assay Buffer/CTSG Assay Buffer	0	9	10
Cathepsin G Inhibitor/CTSG Inhibitor control	0	1	0
CTSG Enzyme Solution	50	50	50
Cathepsin G Substrate/CTSG Substrate Solution	40	40	40

11.6 Measure absorbance on a microplate reader at OD 405nm in a kinetic mode, every 2 – 3 minutes, for at least 1-2 hours at 37°C protected from light.

11.7

***NOTE**: Incubation time depends on the Cathepsin G activity in samples. Longer incubation times may be required if Cathepsin G activity is low.*

We recommend measuring the OD in kinetic mode, and choosing two time points (T_1 & T_2) in the linear range to calculate the Cathepsin G activity of the samples.

12. CALCULATIONS

- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).

12.1 Average the duplicate reading for each test sample compound, Inhibitor Control and Enzyme control.

12.2 Plot the absorbance values for each sample and control as a function of the final concentration of compound.

12.3 Calculate the slope for all samples (S), Inhibition Control and Enzyme Control (EC):

$$Slope = \left(\frac{A2 - A1}{T2 - T1}\right) * D$$

Where:

A_1 = Absorbance value at OD 405 nm at Time T_1.

A_2 = Absorbance value at OD 405 nm at Time T_2.

T_1 = Time of the first reading (A_1) in minutes.

T_2 = Time of the second reading (A_2) in minutes.

D = sample dilution factor.

12.4 Calculate the % Relative inhibitions as follows

$$\%\ Relative\ Inhibition = \frac{Slope\ of\ EC\ - Slope\ of\ S}{Slope\ of\ EC} \times 100$$

13. TYPICAL DATA

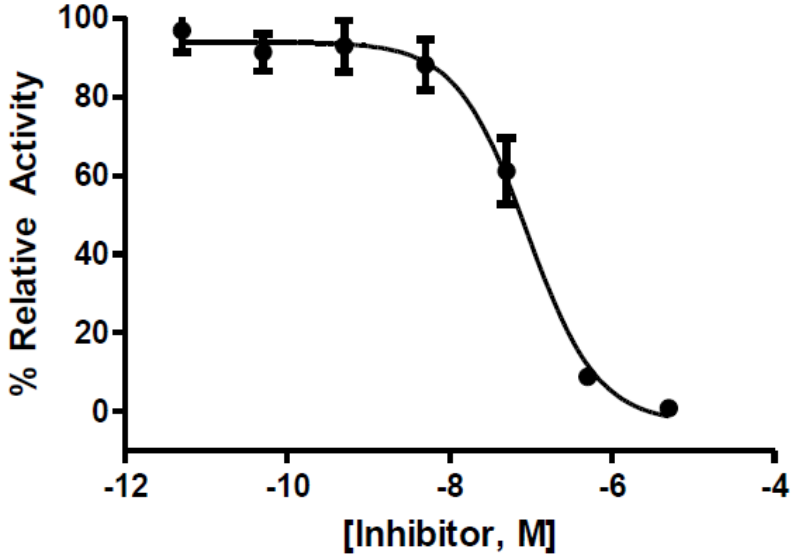


Figure 1. Inhibition of Cathepsin G activity by Cathepsin G Inhibitor/CTSG Inhibitor. Assay was performed following kit protocol.

14. QUICK ASSAY PROCEDURE

***NOTE**: This procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.*

- Prepare enzyme mix, substrate mix, inhibitor (aliquot if necessary) and get equipment ready.
- Prepare samples and dissolve test inhibitors in suitable solvent.
- Prepare enzyme solution for all wells to be set up (50 µL/well)

Component	Enzyme Solution (µL)
Cathepsin G Assay Buffer/CTSG Assay Buffer	**49**
Human Cathepsin G II/Cathepsin G Enzyme	**1**

- Set up plate as follows:

Component	Sample Well (S) (µL)	Solvent control (BC) (µL)	Enzyme Control (EC) (µL)	Inhibitor Control (IC) (µL)
Enzyme Mix	**50**	**50**	**50**	**50**
Solvent test compound	**0**	**10**	**0**	**0**
Test Inhibitor Compound	**10**	**0**	**0**	**0**
Assay Buffer	**0**	**0**	**10**	**9**
Inhibitor control	**0**	**0**	**0**	**1**

- Incubate RT 15 min.
- Prepare 40 µL Cathepsin G Substrate/CTSG Substrate Mix for each well (Number wells + 1).

Component	Substrate Mix (µL)
Cathepsin G Assay Buffer/CTSG Assay Buffer	**38**
Cathepsin G Substrate/CTSG Substrate	**2**

- Add 40 µL of Cathepsin G Substrate/CTSG Substrate Mix to all wells.
- Measure plate on a microplate reader at OD = 405 nm in a kinetic mode at 37°C for 1 – 2 hours

15. TROUBLESHOOTING

Problem	Cause	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at room temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of a different 96-well plate	Colorimetric: Clear plates Fluorometric: black wells/clear bottom plate
Lower/ Higher readings in samples and Standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Allowing reagents to sit for extended times on ice	Always thaw and prepare fresh reaction mix before use
	Incorrect incubation times or temperatures	Verify correct incubation times and temperatures in protocol
Standard readings do not follow a linear pattern	Pipetting errors in standard or reaction mix	Avoid pipetting small volumes (< 5 µL) and prepare a master mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Always refer to dilutions on protocol
Unanticipated results	Measured at incorrect wavelength	Check equipment and filter setting
	Samples contain interfering substances	Troubleshoot if it interferes with the kit
	Sample readings above/ below the linear range	Concentrate/ Dilute sample so it is within the linear range

16. **FAQ**

17. **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)