Cathepsin G Inhibitor Screening Kit (Colorimetric)

(Catalog #BN00442; 100 assays, Store kit at -20°C)

I. Introduction:

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 remodeling at sites of inflammation. Assay Genie's Cathepsin G Inhibitor Screening Kit utilizes 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/characterize potential inhibitors of Cathepsin G.

CTSG Substrate-pNA $\xrightarrow{\text{Cathepsin G}}$ Cleaved substrate + pNA (Absorbance)

CTSG Substrate-pNA $\xrightarrow{\text{Cathepsin G + CTSG inhibitor}}$ Decrease in Absorbance /No Absorbance

II. Applications:

- Screen/study/characterize potential inhibitors of Cathepsin G.

III. Kit Contents:

Components	BN00442	Cap Code	Part Number
CTSG Assay Buffer	20 ml	WM	BN00442-1
Cathepsin G (human)	1 vial	Green	BN00442-2
CTSG Substrate	0.2 ml	Red	BN00442-3
CTSG Inhibitor (0.5 mM)	20 µl	Blue	BN00442-4

IV. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom.
- Multi-well spectrophotometer.

V. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Briefly centrifuge small vials at low speed prior to opening. Read the entire protocol before performing the assay.

- **CTSG Assay Buffer:** Warm CTSG Assay Buffer to room temperature before use.
- **Cathepsin G (human):** Reconstitute in 110 µl of CTSG Assay Buffer. Mix by gently pipetting up & down. Aliquot and store at -80°C. Avoid repeated freeze/thaw.

VI. Cathepsin G Inhibitor Screening Protocol:

1. **Cathepsin G Enzyme Solution Preparation**: For each well, prepare 50 µl of Cathepsin G enzyme solution.

   49 µl CTSG Assay Buffer
   1 µl Cathepsin G

   Mix well and add 50 µl/well into desired wells in a 96-well microtiter plate.

2. **Screening Compounds, Inhibitor Control and Blank Control Preparations:** Dissolve test inhibitors into proper solvent. Dilute to 10X the desired test concentration with CTSG Assay Buffer. Add 10 µl diluted test inhibitors (Sample, S) or CTSG Assay Buffer (Enzyme Control, EC) into Cathepsin G enzyme containing wells. For Inhibitor Control (IC), add 1 µl CTSG Inhibitor and 9 µl CTSG Assay Buffer into Cathepsin G enzyme well(s). Incubate at room temperature for 10-15 min.

3. **Cathepsin G Substrate Preparation:** For each well, prepare 40 µl of the substrate solution.

   38 µl CTSG Assay Buffer
   2 µl CTSG Substrate

   Mix and add 40 µl of Cathepsin G Substrate solution into Enzyme Control, Inhibitor Control, and sample wells. Mix well.

4. **Measurement:** Read absorbance at 405 nm in a kinetic mode for 1-2 hrs. at 37°C. Choose two time points (T_1 and T_2) in the linear range of the plot and obtain the corresponding values for the absorbance (OD_1 and OD_2).

5. **Calculations**: Calculate the slope for all Samples (S), including Enzyme Control (EC), by dividing the net ΔOD (OD_2-OD_1) values with the time ΔT (T_2-T_1).

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

Note: Irreversible inhibitors that inhibit the Cathepsin G activity completely at the tested concentration will have ΔOD = 0 and thus the % Relative Inhibition will be 100%.

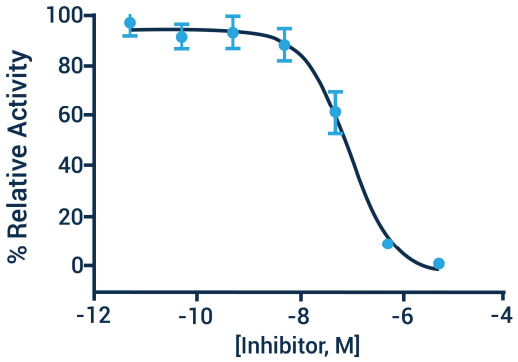

Figure: Inhibition of Cathepsin G activity by CTSG Inhibitor. Assay was performed following kit protocol.

FOR RESEARCH USE ONLY! Not to be used on humans.