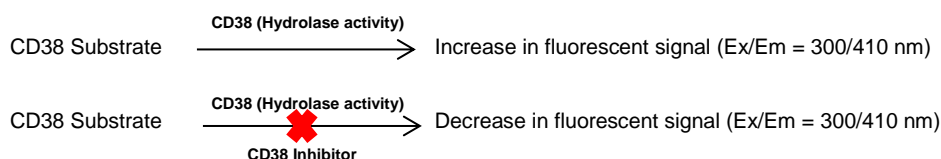


CD38 (Hydrolase) Inhibitor Screening Kit (Fluorometric)

04/21

(Catalog # K2086-100; 100 assays; Store at -20 °C)

- I. **Introduction:** Cluster of differentiation 38 (CD38), also known as cyclic ADP ribose hydrolase is a type II transmembrane glycoprotein that can function either as a receptor or as an enzyme. It is found on the surface of many immune cells, including plasma B cells, natural killer cells, CD4⁺, CD8⁺ etc. It is a multifunctional enzyme involved in cell-adhesion, calcium signaling and Nicotinamide Adenine Dinucleotide (NAD⁺) metabolism. The hydrolase activity of CD38 helps maintain the appropriate levels of NAD⁺ for all NAD⁺ dependent metabolic processes to occur. Elevated levels of CD38 are associated with aging, obesity, diabetes, heart disease, asthma, inflammation and tumorigenesis etc. **BioVision's CD38 (Hydrolase) Inhibitor Screening Kit** is a plate-based fluorometric assay designed to screen, study and characterize potential inhibitors of CD38 hydrolase activity. The assay utilizes a selective CD38 substrate to generate a fluorescent signal measured at Ex/Em = 300/410 nm. In the presence of potential CD38 hydrolase inhibitors, the fluorescent signal is reduced. The assay is quick, easy, and sensitive for high-throughput screening of CD38 inhibitors. Additionally, the kit includes a CD38 Inhibitor as a control inhibitor.



II. Application:

- Screening or characterizing CD38 (Hydrolase) inhibitors.

III. Kit Contents:

Components	K2086-100	Cap Code	Part Number
CD38 Assay Buffer	25 ml	WM	K2086-100-1
CD38 Substrate	50 μ l	Amber	K2086-100-2
CD38, Human Recombinant	1 vial	Blue	K2086-100-3
CD38 Inhibitor (5 mM)	50 μ l	Yellow	K2086-100-4

IV. User Supplied Reagents and Equipment:

- 96-well white plate with flat bottom
- Multi-well spectrophotometer

V. Storage Conditions and Reagent Preparation:

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

- **CD38 Assay Buffer:** Store at 4 °C or -20 °C. Bring to room temperature (RT) before use.
- **CD38 Substrate:** Store at -20 °C. Divide into aliquots and store on ice while in use.
- **CD38, Human Recombinant:** Reconstitute the vial in 30 μ l CD38 Assay Buffer. Divide into aliquots and store at -20 °C. Keep on ice during use. Avoid repeated freeze-thaw cycles.
- **CD38 Inhibitor (5 mM in DMSO):** Warm to RT. Divide into aliquots and store at -20 °C.

VI. CD38 Inhibitor Screening Protocol:

1. **CD38, Human Recombinant Enzyme Dilution:** Prepare 1:40 dilution of the CD38 enzyme using CD38 Assay Buffer. Mix thoroughly and keep on ice. Add 10 μ l of diluted CD38 enzyme into the desired wells of a 96-well white plate labeled as **Sample**, **Solvent Control**, **Inhibitor Control** and **Enzyme Control**. Adjust the volume of all wells to 25 μ l using CD38 Assay Buffer.

2. **Screening Test Inhibitor(s):** Dissolve Test Inhibitor(s) in an appropriate solvent to make 100X stock solution. Dilute the stock Test Inhibitor to 4X using CD38 Assay Buffer. Add 25 μ l of diluted Test Inhibitor into the **Sample** well(s). Add 25 μ l of 4X Solvent (4X final well solvent concentration) into the **Solvent Control** well. **Note:** Solvents used to solubilize the Test Inhibitor(s) might affect the enzymatic activity. Thus, prepare a **Solvent Control** well by adding 25 μ l of solution with the same final concentration of solvent in assay buffer that is used to dissolve the Test Inhibitor(s).

3. **Enzyme Control, Background Control and Inhibitor Control Preparation:** Add 25 μ l of CD38 Assay Buffer to the **Enzyme Control** well. For **Background Control**, add 50 μ l of CD38 Assay Buffer in a separate well. To the **Inhibitor Control** well, add 2 μ l of 5 mM CD38 Inhibitor and adjust the volume to 50 μ l/well by adding 23 μ l CD38 Assay Buffer. At this stage, the volume of all wells including Sample, Solvent Control, Inhibitor Control, Enzyme Control and Background Control is 50 μ l/well.

IC₅₀ estimation (Optional): Prepare several dilutions of the Test Inhibitor(s) in CD38 Assay Buffer while maintaining the consistent final Solvent Concentration in all wells. Add 25 μ l of each dilution into the designated wells.

4. CD38 Substrate Mix Preparation: Mix enough CD38 Substrate Mix for the number of assays to be performed. For each well, prepare 50 μ l CD38 Substrate Mix containing

	<u>CD38 Substrate Mix</u>
CD38 Assay Buffer	49.5 μ l
CD38 Substrate	0.5 μ l

Add 50 μ l CD38 Substrate Mix to Sample, Solvent Control, Inhibitor Control, Enzyme Control and Background Control wells and mix well. The total reaction volume is 100 μ l/well.

5. Measurement: Allow the plate to warm up at 37 °C in the plate reader before measuring the fluorescence in kinetic mode (Ex/Em = 300/410 nm) for 30-60 min. Choose any two time points (t_1 & t_2) in the linear range of the plot and obtain the corresponding RFU values.

Note: The Enzyme progressive curve is hyperbolic, with an initial linear portion followed by progressively slower reaction. Use the initial portion to check the linear range of the reaction.

6. Calculation: Subtract the RFU of the BC well from all Test Inhibitor(s) [S], Enzyme Control [EC], Solvent Control [SC] and Inhibitor Control [IC] wells. Obtain Δ RFU for S, EC, SC and IC by subtracting RFU at time t_1 from RFU at time t_2 , such that t_2 and t_1 is within a linear range of the assay. If Δ RFU of Solvent Control [SC] is significantly different from Δ RFU of Enzyme Control [EC], use its values to determine the effect of test inhibitor.

Calculate the relative % inhibition of the Test Inhibitor(s) as below:

$$\% \text{ Relative Inhibition} = \frac{\Delta\text{RFU}[\text{EC}] - \Delta\text{RFU}[\text{S}]}{\Delta\text{RFU}[\text{EC}]} \times 100$$

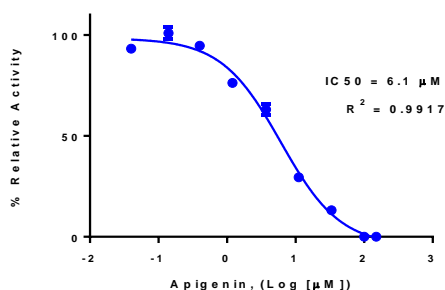


Figure: Inhibition of CD38 activity by Apigenin. IC_{50} was calculated to be $6.1 \pm 0.1 \mu$ M. Assay was performed following the kit protocol.

VII. Related Products:

Human CellExp™ CD38, human recombinant (Cat. # P1014-10, 50)
 Human CellExp™ CD38, Mouse Recombinant (Cat. # PP1338-10, 50)
 CD38 (Cyclase) Activity Assay Kit (Fluorometric) (Cat. # K2042-100)
 NAD⁺/NADH Quantification Colorimetric Kit (Cat. # K337-100)
 PicoProbe™ NADH Fluorometric Assay Kit (Cat. # K338-100)
 EZScreen™ NAD⁺/NADH Colorimetric Assay Kit (384-well) (Cat. # K958-400)
 Apigenin (Cat. # 2508-25, 100)
 Quercetin, Dihydrate (Cat. # 1773-250, 1000)

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