

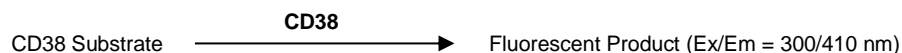
CD38 (Cyclase) Activity Assay Kit (F)

rev 04/21

(Catalog # K2042-100; 100 assays; Store at -20 °C)

I. Introduction:

Cluster of differentiation 38 (CD38), also known as cyclic ADP ribose hydrolase is a multifunctional enzyme that catalyzes the synthesis and hydrolysis of Nicotinamide Adenine Dinucleotide (NAD) to Nicotinamide and ADP-ribose (ADPR). Additionally, CD38 generates second messengers, cyclic ADP-ribose using NAD as the substrate. Due to its role in NAD⁺ metabolism, the study of CD38 and its functions has been of great importance. CD38 has been implicated in regulating NAD⁺ metabolism, aging, and other diseased conditions including obesity, diabetes, heart disease, asthma, inflammation etc. Furthermore, CD38 is expressed by lymphoid, myeloid, red blood cells and platelets, the highest expression being found in plasma cells. High expression of CD38 by plasma cells makes CD38 an ideal target for targeted immunotherapy. **BioVision's CD38 (Cyclase) Activity Assay Kit** can be used to detect its activity in biological samples. The kit utilizes the ability of active CD38 to catalyze the conversion of a CD38 substrate to a fluorescent product (Ex/Em = 300/410 nm). This assay kit provides a rapid, simple and sensitive method to detect CD38 activity in a variety of sample types.



II. Applications:

- Measurement of CD38 activity in various tissues/cells
- Mechanistic studies of various cancers
- Screening anti-cancer drugs

III. Sample Types:

- Animal tissues: Soft tissues (i.e. Spleen)
- Cultured cells: Adherent or suspension cells. Highest expression of the protein was found in B-cells

IV. Kit Contents:

Components	K2042-100	Cap Code	Part Number
CD38 Assay Buffer	25 ml	WM	K2042-100-1
CD38 Lysis Buffer	25 ml	NM	K2042-100-2
CD38 Substrate	1 vial	White	K2042-100-3
CD38 Positive Control	1 vial	Orange	K2042-100-4
CD38 Standard	20 μ l	Yellow	K2042-100-5

V. User Supplied Reagents and Equipment:

- dH₂O
- 96-well white plate with flat bottom
- Temperature-controlled spectrophotometer (plate reader)

VI. Storage Conditions and Reagent Preparation:

Upon arrival, store the 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 and CD38 Lysis Buffer:** Warm to room temperature (RT) before use. Store at 4 °C.
- **CD38 Substrate:** Reconstitute with 220 μ l dH₂O. Divide into aliquots and store at -20 °C. Keep on ice while in use.
- **CD38 Positive Control:** Reconstitute the vial with 100 μ l CD38 Assay Buffer. Divide into aliquots and store at -20 °C. Keep on ice while in use. Avoid repeated freeze-thaw cycles. Use within six months after reconstitution.
- **CD38 Standard (10 mM):** Warm to RT before use. Store at 4 °C.

VII. CD38 Activity Assay Protocol:

1. Sample Preparation: Homogenize tissue (10 mg) or cells (1×10^6) in 200 μ l ice cold CD38 Lysis Buffer on ice. Centrifuge at 10000 x g and 4 °C for 10 min to remove cell debris and save the supernatant. Prepare a well for each sample to be tested labeled as **Sample**. Add 1-50 μ l of the sample supernatant into the well of a 96 well white plate with flat bottom. Bring the volume to 50 μ l/well with CD38 Assay Buffer. Prepare a **Blank Control well** by adding 50 μ l of CD38 Assay Buffer.

Note: For Unknown Samples, we suggest testing several doses of your sample to make sure the readings are within the Standard Curve range.

2. Standard Curve Preparation: Dilute 10 mM stock CD38 Standard to 0.1 mM by adding 10 μ l of 10 mM stock CD38 Standard to 990 μ l CD38 Assay Buffer, mix well. Further dilute the 0.1 mM CD38 Standard to 10 μ M CD38 Standard by adding 100 μ l of 0.1 mM CD38 Standard to 900 μ l of CD38 Assay Buffer. Add 0, 4, 8, 12, 16 & 20 μ l of the 10 μ M CD38 Standard solution into a series of wells in the 96 well white plate. Adjust volume to 50 μ l/well with CD38 Assay Buffer resulting in 0, 40, 80, 120, 160, and 200 pmol/well of the CD38 Standard.

3. CD38 Positive Control: Add 2 to 5 μ l of the reconstituted CD38 Positive Control into desired well(s). Adjust the volume to 50 μ l/well with CD38 Assay Buffer.

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

	<u>Reaction Mix</u>	<u>Standard Mix</u>
CD38 Assay Buffer	47.6 μ l	50 μ l
CD38 Substrate	2.4 μ l	----

Mix well. Add 50 μ l of Reaction Mix into the Positive Control and Sample(s) wells and 50 μ l of Standard Mix into the Standard wells respectively.

5. Measurement: Measure the fluorescence (Ex/Em = 300/410 nm) in kinetic mode for 30-60 min at 37 °C.

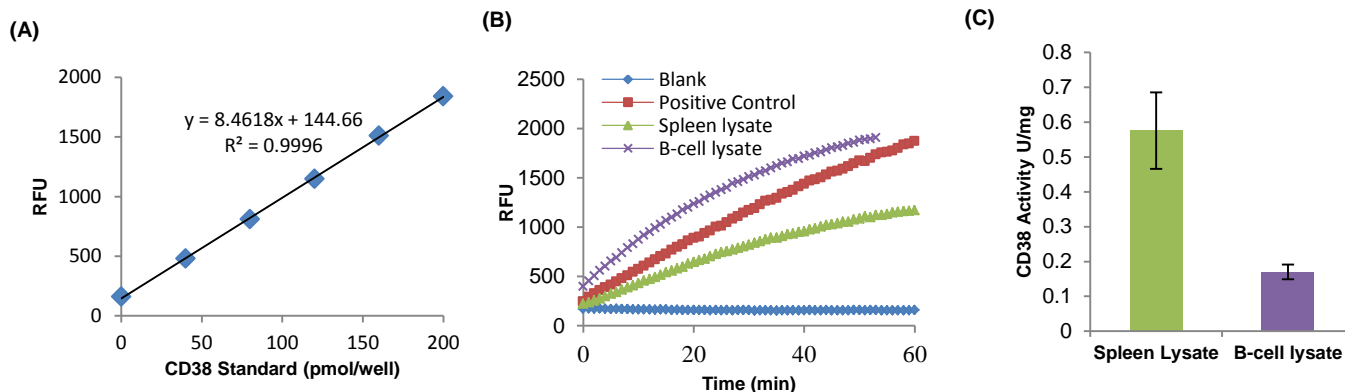
Note: Incubation time depends on the CD38 Activity in samples. We recommend measuring the fluorescence in a kinetic mode, and choosing any two time points (T_1 & T_2) in the linear range of the curve. The Standard Curve can be read in Endpoint mode (at the end of 30-60 min incubation).

6. Calculation: Subtract 0 Standard reading from all Standard readings. Plot the CD38 Standard Curve. Subtract Blank Control readings from the Sample(s) to get the corrected Sample reading. Apply the corrected Sample reading to the CD38 Standard Curve to get B nmol of product generated during the reaction time ($\Delta T = T_2 - T_1$). To determine the activity of CD38 in the sample(s), use the following equation:

$$\text{Sample CD38 Activity} = \left\{ \frac{B}{\Delta T \times P} \right\} \times DF = \text{nmol/min/mg} = \text{U/mg}$$

Where,

- B** = Amount of product from the Standard Curve (nmol)
- ΔT** = Difference between T_2 and T_1 (min)
- P** = Amount of protein in the Sample (mg)
- DF** = Sample Dilution factor (D= 1 for undiluted samples)



Figures: (A) CD38 Standard Curve. (B) Reaction Kinetic curve of CD38 Positive Control, spleen lysate and B-cell lysate. (C) CD38 activity assayed in rat spleen lysate (2 μ g) and B-cell lysate (2.7 μ g). Assays were performed following the kit protocol.

VIII. Related Products:

NAD/NADH Quantitation Colorimetric Kit (Cat# K337)
 PicoProbe™ NADH Fluorometric Assay Kit (Cat# K338)
 EZScreen™ NAD⁺/NADH Colorimetric Assay Kit (384-well) (Cat# K958)
 β -Nicotinamide adenine dinucleotide sodium salt (Cat# 9457)
 Human CellExp™ CD38, Mouse Recombinant (Cat# P1335)
 Human CellExp™ CD38, human recombinant (Cat# P1014)

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