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CD38 (Hydrolase) Activity Assay Kit (Fluorometric)

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(Catalog # K2095-100; 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) Activity Assay Kit** provides an easy, quick and sensitive method to detect CD38 (hydrolase) activity in various sample types. The kit utilizes the ability of active CD38 to catalyze the conversion of a selective CD38 substrate to a fluorescent product measured at Ex/Em = 300/410 nm.

II. Applications:
- Measurement of CD38 (Hydrolase) activity in various biological samples
- Mechanistic studies of various cancers

III. Sample Types:
- Animal tissues: (i.e. liver, spleen etc.)

IV. Kit Contents:

Components	K2095-100	Cap Code	Part Number
CD38 Assay Buffer	25 ml	WM	K2095-100-1
CD38 Lysis Buffer	25 ml	NM	K2095-100-2
CD38 Substrate	50 µl	Amber	K2095-100-3
CD38 Positive Control	1 vial	Blue	K2095-100-4
CD38 Standard	20 µl	Yellow	K2095-100-5

V. User Supplied Reagents and Equipment:
- dH_2O
- 96-well white plate with flat bottom
- Multi-well spectrophotometer

VI. 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 and CD38 Lysis Buffer:** Store at 4 °C. Bring to room temperature (RT) before use.
- **CD38 Substrate:** Store at -20 °C. Divide into aliquots and keep on ice while in use.
- **CD38 Positive Control:** Reconstitute the vial with 100 µl CD38 Assay Buffer. Divide into aliquots and store at -20 °C. Keep on ice during use. Avoid repeated freeze-thaw cycles.
- **CD38 Standard (10 mM):** Warm to RT before use. Store at 4 °C.

VII. CD38 (Hydrolase) Activity Assay Protocol:

1. Sample Preparation: Homogenize tissue (25 mg) in 250 µl ice cold CD38 Lysis Buffer on ice. Centrifuge at 14,000 *x g* and 4 °C for 10 min to remove the cell debris and save the supernatant for the assay. Keep all samples on ice during the assay. Prepare a well for each sample to be tested labeled as **Sample**. Add 1- 50 µl of the sample supernatant into the wells of a 96 well white plate with flat bottom. Adjust the volume to 50 µl/well with CD38 Assay Buffer. Prepare a **Blank Control** well by adding 50 µl of CD38 Assay Buffer. **Note:** For Unknown Samples, we suggest testing several doses of the sample to make sure the readings are within the Standard Curve range.

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

3. CD38 Positive Control: Add 2 - 5 µl of reconstituted CD38 Positive Control into the desired wells. Adjust the volume to 50 µl/well using CD38 Assay Buffer. At this stage, all wells including Sample(s), CD38 Positive Control and Blank Control contain 50 µl/well.

4. CD38 Substrate Mix Preparation: Mix enough CD38 Substrate Mix for the number of assays to be performed. Prepare 50 µl Substrate Mix per reaction as shown below.

	CD38 Substrate Mix
CD38 Assay Buffer	49.5 µl
CD38 Substrate	0.5 µl

Mix well. Add 50 µl Substrate Mix to Sample(s), CD38 Positive Control and Blank Control wells. The total reaction volume is 100 µl/well.

5. Measurement: Measure the fluorescence at Ex/Em = 300/410 nm in kinetic mode for 30-60 min at 37 °C. **Notes:** Incubation time depends on the CD38 Activity in samples. We recommend measuring the fluorescence in kinetic mode, and choosing any two time points (T_1 & T_2) in the linear range of the curve. The Standard well can be read for 10 min in kinetic mode.

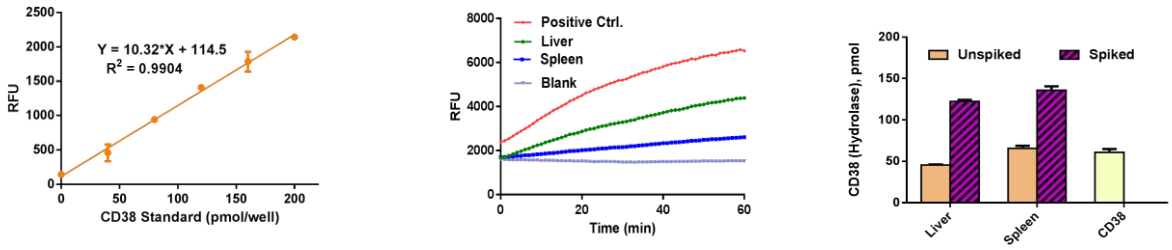
6. Calculation: Subtract the 0 Standard reading from all Standard readings and plot the CD38 Standard Curve. Subtract the Blank Control from the Sample(s) readings to get the corrected Sample reading. Apply the corrected Sample readings to the CD38 Standard Curve to get B pmol of product generated during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

$$\textbf{Sample CD38 (Hydrolase) Activity} = \frac{B}{\Delta T \times P} \times D = \text{pmol/min/}\mu\text{g} = \text{mU/mg}$$

Where,

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

(A) **(B)** **(C)**



Figures: (A) CD38 Standard Curve. (B) Kinetic activity of CD38 (hydrolase) Positive Control and rat samples including spleen lysate & liver lysate (20 µg). (C) CD38 (hydrolase) activity using rat samples spiked with 60 pmol CD38 Positive Control. The average spike recoveries were 107 % and 97 % respectively. Assay was performed following the kit protocol.

VIII. Related Products:

Human CellExp™ CD38, Human Recombinant (Cat. # P1014-10, 50)
Human CellExp™ CD38, Mouse Recombinant (Cat. # P1338-10, 50)
CD38 (Cyclase) Activity Assay Kit (Fluorometric) (Cat. # K2042-100)
CD38 (Hydrolase) Inhibitor Screening Kit (Fluorometric) (Cat. # K2086-100)
NAD/NADH Fluorometric Assay Kit (Cat. # K338-100)
Uric Acid Colorimetric/Fluorometric Assay Kit II (Cat. # K608-100)
Uricase Activity Assay Kit (Fluorometric) (Cat. # K734-100)
Ammonia Colorimetric Assay Kit (Cat. # K370-100)
Ammonia Colorimetric Assay Kit II (Cat. # K470-100)
Urease Activity Assay Kit (Colorimetric) (Cat. # K378-100)
Urease Inhibitor Screening Kit (Colorimetric) (Cat. # K2079-100)

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