

## ab308274 – CD38 (Hydrolase) Activity Assay Kit (Fluorometric)

Measurement of CD38 (Hydrolase) activity in various biological samples.

For research use only - 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.

For overview, typical data and additional information please visit: [abcam website](#)

### Storage and Stability

Store kit at -20 °C, protected from light. Briefly centrifuge all small vials prior to opening.

### Materials Supplied

Item	Quantity	Storage Condition
CD38 Assay Buffer	25 ml	4 °C
CD38 Lysis Buffer	25 ml	4 °C
CD38 (Hydrolase) Substrate/CD38 Substrate (Avoid light)	50 µl	-20 °C
Active Human CD38/CD38 Positive Control	1 vial	-20 °C
CD38 Standard (Avoid light)	20 µl	-20 °C

### Materials Required, Not Supplied

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

- dH<sub>2</sub>O
- 96-well white plate with flat bottom
- Multi-well spectrophotometer

### Reagent Preparation

CD38 Assay Buffer and CD38 Lysis Buffer: Store at 4 °C. Bring to room temperature (RT) before use.

CD38 (Hydrolase) Substrate/CD38 Substrate: Store at -20 °C. Divide into aliquots and keep on ice while in use.

Active Human CD38/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.

### CD38 (Hydrolase) Activity Assay Protocol

#### 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.

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

#### 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 and 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.

#### CD38 Positive Control:

Add 2 - 5 µl of reconstituted Active Human CD38/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), Active Human CD38/CD38 Positive Control and Blank Control contain 50 µl/well.

#### 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 (Hydrolase) Substrate/CD38 Substrate	0.5 µl

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

### Measurement

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

**Δ Note:** Incubation time depends on the CD38 Activity in samples. We recommend measuring the fluorescence in kinetic mode and choosing any two time points (*T*<sub>1</sub> and *T*<sub>2</sub>) in the linear range of the curve. The Standard well can be read for 10 min in kinetic mode.

### 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 (Δ*T* = *T*<sub>2</sub> - *T*<sub>1</sub>). To determine the activity of CD38 in the sample(s), use the following equation:

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

Where, **B** = Amount of product from the Standard Curve (pmol)

**Δ*T*** = Difference between *T*<sub>2</sub> and *T*<sub>1</sub> (min)

- P** = Amount of protein in the Sample ( $\mu\text{g}$ )  
**D** = Sample Dilution factor (D = 1 for undiluted samples)

### Technical Support

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