ab284540 – CD38 Activity Assay Kit (Fluorometric)

For the detection of CD38 activity in biological samples. For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab284540

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.

Storage and Stability

On receipt entire assay kit should be stored at -20°C, protected from light. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Materials Supplied

Item	Quantity	Storage Condition
CD38 Assay Buffer	25 mL	-20°C
CD38 Lysis Buffer	25 mL	-20°C
CD38 Positive Control	1 vial	-20°C
CD38 Standard	20 µL	-20°C
CD38 (Cyclase) Substrate/CD38 Substrate	1 vial	-20°C

Materials Required, Not Supplied

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

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

Reagent Preparation

- Briefly centrifuge all small vials prior to opening

CD38 Assay Buffer and CD38 Lysis Buffer: Warm to room temperature (RT) before use. Store at 4 °C.
CD38 (Cyclase) Substrate/CD38 Substrate: Reconstitute with 220 µL dH2O. Divide into aliquots and store at -20 °C. 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 while in use. Avoid repeated freeze-thaw cycles. Use within six months after reconstitution.
CD38 Standard: Warm to RT before use. Store at 4 °C

Assay Protocol

Sample preparation:

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

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

Standard Curve Preparation:

1. Dilute 10 mM stock CD38 Standard to 0.1 mM by adding 10 µL of 10 mM stock CD38 Standard to 990 µL CD38 Assay Buffer, mix well.
2. 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.
3. Add 0, 4, 8, 12, 16 & 20 µL of the 10 µM CD38 Standard solution into a series of wells in the 96 well white plate.
4. Adjust volume to 50 µL/well with CD38 Assay Buffer resulting in 0, 40, 80, 120, 160, and 200 pmol/well of the CD38 Standard.

Positive control

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

Reaction Mix:

1. Mix enough reagents for the number of assays to be performed. For each well, prepare 50 µL Mix containing:

	Reaction Mix	Standard Mix
CD38 Assay Buffer	47.6 µL	50 µL
CD38 (Cyclase) Substrate/CD38 Substrate	2.4 µL	-

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

Measurement

Measure the fluorescence (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 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).*

Calculation:

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

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

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)

Technical Support

For all technical or commercial enquiries please go to:

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