ab308152 – CD38 (Cyclase) Inhibitor Screening Kit (Fluorometric)

A 96-well plate based colorimetric assay to measure the activation of human c-Fos in nuclear extracts or cell lysates.
For research use only - not intended for diagnostic use.

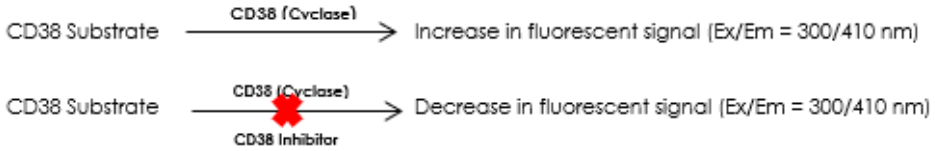
For overview, typical data and additional information please visit:

http://www.abcam.com/ab308152

Storage and Stability
The entire Assay kit may be stored at -20 °C and protected from light.

Introduction:

Cluster of differentiation 38 (CD38), also known as cyclic ADP ribose hydrolase or ADP-ribosyl cyclase 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. It is found on the surface of many immune cells, including plasma B cells, natural killer cells, CD4+, CD8+, etc. Elevated levels of CD38 are associated with aging, obesity, diabetes, heart disease, asthma, inflammation and tumorigenesis etc. **The CD38 (Cyclase) Inhibitor Screening Kit** is a plate-based fluorometric assay designed to screen, study and characterize potential inhibitors of CD38 cyclase 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 cyclase 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.



Materials Supplied

Item	Quantity
CD38 Assay Buffer	25 mL
CD38 Substrate	1 vial
CD38, Human Recombinant	1 vial
CD38 Inhibitor	50 µl

Materials Required, Not Supplied

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

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

Reagent Preparation

- 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: Store at 4 °C or -20 °C. Bring to room temperature (RT) before use.

CD38 Substrate: Reconstitute the vial in 220 µl dH_2O. Divide into aliquots and store at -20 °C. Keep on ice during use.

CD38, Human Recombinant: Reconstitute the vial in 110 µl 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.

Sample Preparation

CD38, Human Recombinant Enzyme Dilution: Prepare 1:5 dilution of the CD38 enzyme using CD38 Assay Buffer. Mix thoroughly and keep on ice. Add 2.5 µl 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 µl using CD38 Assay Buffer.

Screening Test Inhibitor(s): Dissolve the 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 µl of diluted Test Inhibitor into the **Sample** well(s). Add 25 µl 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 µl of solution with the same final concentration of solvent in assay buffer that is used to dissolve the Test Inhibitor(s).

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

Assay Procedure

1. **CD38 Substrate Mix Preparation**: Mix enough CD38 Substrate Mix for the number of assays to be performed. For each well, prepare 50 µl CD38 Substrate Mix containing

	CD38 Substrate Mix
CD38 Assay Buffer	48 µl
CD38 Substrate	2 µl

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

2. **Measurement**: Measure the fluorescence in kinetic mode (Ex/Em = 300/410 nm) at 37 °C 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.
3. **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 ΔRFU for S, EC, SC and IC by subtracting RFU at time t1 from RFU at time t2, such that t2 and t1 is within a linear range of the assay. If ΔRFU of Solvent Control [SC] is significantly different from ΔRFU of Enzyme Control [EC], use its values to determine the effect of test inhibitors dissolved in the same solvent.

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

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

For technical support contact information, visit: www.abcam.com/contactus