

## AB308270 – CD38 (Hydrolase) Inhibitor Screening Kit (Fluorometric)

Screening or characterizing CD38 (Hydrolase) inhibitors.

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. Read the entire protocol before performing the assay.

### Materials Supplied

Item	Quantity	Storage Condition
CD38 Assay Buffer	25 ml	4 °C or -20 °C
CD38 (Hydrolase) Substrate/CD38 Substrate (Avoid light)	50 µl	-20 °C
Human CD38/CD38, Human Recombinant	1 vial	-20 °C
CD38 Inhibitor (Apigenin)/CD38 Inhibitor (5 mM) (Avoid light)	50 µl	-20 °C

### 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

CD38 Assay Buffer: Store at 4 °C or -20 °C. Bring to room temperature (RT) before use.

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

Human CD38/CD38, Human Recombinant: Reconstitute the vial in 30 µl CD38 Assay Buffer. Divide into aliquots and store at -20 °C. Keep on ice during use. Avoid repeated freeze-thaw cycles.

CD38 Inhibitor (Apigenin)/CD38 Inhibitor (5 mM in DMSO): Warm to RT. Divide into aliquots and store at -20 °C.

### CD38 Inhibitor Screening Protocol:

#### CD38, Human Recombinant Enzyme Dilution:

Prepare 1:40 dilution of the Human CD38/CD38 enzyme using CD38 Assay Buffer. Mix thoroughly and keep on ice. Add 10 µl of diluted Human CD38/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 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.

**Δ 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 (Apigenin)/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.

### IC<sub>50</sub> 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.

### CD38 (Hydrolase) Substrate/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	49.5 µl
CD38 (Hydrolase) Substrate/CD38 Substrate	0.5 µl

Add 50 µl CD38 (Hydrolase) Substrate/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.

### Measurement

Allow the plate to warm up at 37 °C in the plate reader before measuring the fluorescence in kinetic mode (Ex/Em = 300/410 nm) 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.

**Δ Note:** *The Enzyme progressive curve is hyperbolic, with an initial linear portion followed by progressively slower reaction. Use the initial portion to check the linear range of the reaction.*

### 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  $\Delta$ RFU for S, EC, SC and IC by subtracting RFU at time  $t_1$  from RFU at time  $t_2$ , such that  $t_2$  and  $t_1$  is within a linear range of the assay. If  $\Delta$ RFU of Solvent Control [SC] is significantly different from  $\Delta$ RFU of Enzyme Control [EC], use its values to determine the effect of test inhibitor.

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

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

### Technical Support

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