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Data Sheet

CD38 Inhibitor Screening Assay Kit (Cyclase Activity)

Catalog # 71275

BACKGROUND : CD38, a differentiation antigen of B lymphocytes, is a type II integral membrane protein. It is also known as ADP-ribosyl cyclase and nicotinamide adenine dinucleotide (NAD⁺) glycohydrolase. Through its production of cyclic ADP-ribose, CD38 modulates calcium-mediated signal transduction in various cells, including pancreatic β cells. CD38 is a prognostic biomarker for acute B lymphoblastic leukemia.

DESCRIPTION: The *CD38 Inhibitor Screening Assay Kit (Cyclase Activity)* is designed to measure the cyclase activity of CD38 for screening and profiling applications. The CD38 assay kit comes in a convenient 96-well format, with purified recombinant CD38 enzyme, its substrate nicotinamide guanine dinucleotide (NGD⁺), and CD38 assay buffer for 100 enzyme reactions. In addition, the kit includes the CD38 inhibitor quercetin for use as a positive control.

COMPONENTS:

Catalog #	Reagent	Amount	Storage	
71277	CD38, His-Tag (Human), HiP™	25 μ g	-80°C	<i>Avoid multiple freeze/thaw cycles!</i>
	3x CD38 assay buffer	4 ml	-20°C	
	CD38 substrate NGD ⁺	50 μ l	-20°C	
	Quercetin (50 mM DMSO)	100 μ l	-20°C	
79685	Black 96-well plate	1	Room Temp.	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:

Adjustable micropipettor and sterile tips
Fluorescent microplate reader
Rotating or rocker platform

APPLICATIONS: Great for studying enzyme kinetics and screening small molecular inhibitors for drug discovery and HTS applications.

STABILITY: Up to 6 months from date of receipt, when stored as recommended.

REFERENCE: Wei, W., *et al.*, *World J. Biol. Chem.* 2014 **5**(1):58-67.

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ASSAY PROTOCOL:

All samples and controls should be tested in duplicate.

1. Thaw **3x CD38 assay buffer** on ice.
2. Prepare the master mixture (20 μ l per well): N wells x (10 μ l **3x CD38 assay buffer** + 10 μ l water). Add 20 μ l to every well.

	Positive Control	Test Inhibitor	Blank
3x CD38 assay buffer	10 μ l	10 μ l	10 μ l
Water	10 μ l	10 μ l	10 μ l
Test Inhibitor	-	10 μ l	-
Inhibitor Buffer (no inhibitor)	10 μ l	-	10 μ l
1x CD38 assay buffer	-	-	15 μ l
CD38 (16.7 ng/ μ l)	15 μ l	15 μ l	-
NGD ⁺ (diluted)	5 μ l	5 μ l	5 μ l
Total	50 μ l	50 μ l	50 μ l

3. Add 10 μ l of Inhibitor solution of each well labeled as "Test Inhibitor". For the wells labeled "Positive Control" and "Blank", add 10 μ l of the same solution without inhibitor (Inhibitor buffer).
4. Prepare **1x CD38 assay buffer** by diluting **3x CD38 assay buffer** with water. Dilute only enough buffer required for the assay. Store remaining **3x CD38 assay buffer** at -20°C in single-use aliquots. For 100 reactions, prepare 6 ml **1x CD38 assay buffer** by mixing 2 ml of **3x CD38 assay buffer** with 4 ml water.
5. To the wells designated as "Blank", add 15 μ l of **1x CD38 assay buffer**.
6. Thaw **CD38** enzyme on ice. Upon first thaw, briefly spin tube containing enzyme to recover full contents of the tube. Calculate the amount of **CD38** required for the assay and dilute enzyme to 16.7 ng/ μ l with **1x CD38 assay buffer** (250 ng/well). Aliquot remaining **CD38** enzyme into single-use aliquots. Store remaining undiluted enzyme in aliquots at -80°C. *Note: CD38 enzyme is sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*
7. Add 15 μ l of diluted **CD38** enzyme to the wells designated "Positive Control" and "Test Inhibitor Control". Cover the plate and incubate 1 hour at room temperature with slow shaking.

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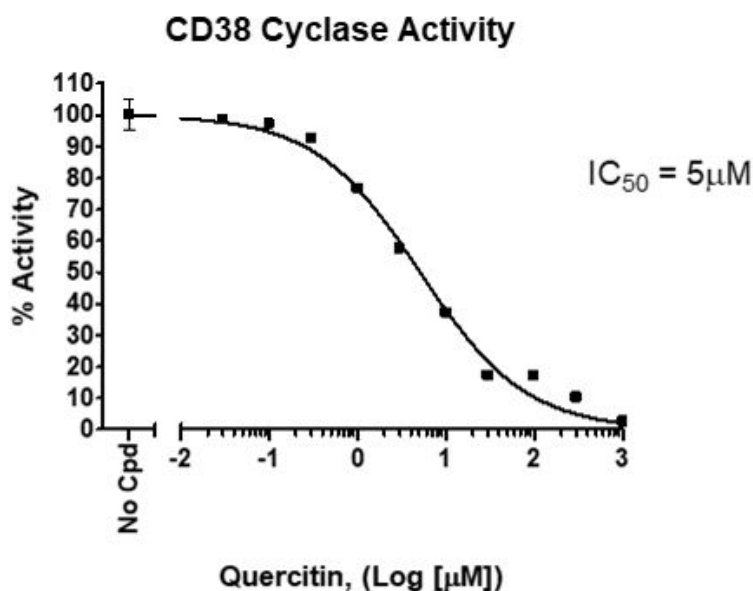
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8. During incubation, dilute **NGD⁺** 10-fold with **1x CD38 assay buffer**. Dilute only the amount required for the assay. Store remaining **NGD⁺** at -20°C in single use aliquots. Discard any unused diluted **NGD⁺** after use.
9. After the 1 hour incubation, remove the plate and add 5 µl of diluted **NGD⁺**.
10. Place plate into plate-reading fluorimeter and prepare to measure.
11. After 4-6 minutes, measure the plate using a fluorimeter capable of excitation at 300 nm and detection of emitted light at 410 nm. The "Blank" value is subtracted from all other values.

Example of Assay Results:



CD38 inhibition by quercetin, measured using the **CD38 Inhibitor Screening Assay Kit (cyclase activity)**, BPS Bioscience Cat. # 71275. Fluorescence was measured using a Bio-Tek microplate reader. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

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RELATED PRODUCTS:

<u>Product Name</u>	<u>Catalog #</u>	<u>Size</u>
CD38, His-Tag (Human), HiP™	71227	100 µg
5'-Nucleotidase/CD73, His-tag	71184	50 µg
Quercetin	27214	5 g
CD73 Inhibitor Screening Assay Kit	72055	96 rxns
CD73 Inhibitor Screening Assay Kit	72058	384 rxns
Adenosine Deaminase (ADA), His-tag	70016	100 µg
NAD+, Biotin-Labeled	80610	500 µl
NAMPT (PBEF1)	71098	50 µg
NAMPT (PBEF1)	91004	50 µg
NMNAT, His-tag	71090	100 µg
TCF/LEF Reporter Kit	60500	500 rxns
TCF/LEF reporter-HEK293 cell line	60501	2 vials

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