6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

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 quercitin for use as a positive control.

COMPONENTS:

Catalog #	Reagent	Amount	Storage	
71277	CD38, His-Tag (Human), HiP™	25 µg	-80°C	***Avoid multiple freeze/thaw cycles!***
	3x CD38 assay buffer	4 ml	-20°C	
	CD38 substrate NGD^+	50 µl	-20°C	
	Quercitin (50 mM DMSO)	100 µl	-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 µl per well): N wells x (10 µl **3x CD38 assay buffer** + 10 µl water). Add 20 µl to every well.

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

3. Add 10 µl of Inhibitor solution of each well labeled as “Test Inhibitor”. For the wells labeled “Positive Control" and “Blank”, add 10 µl 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 µl 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/µl 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 µl 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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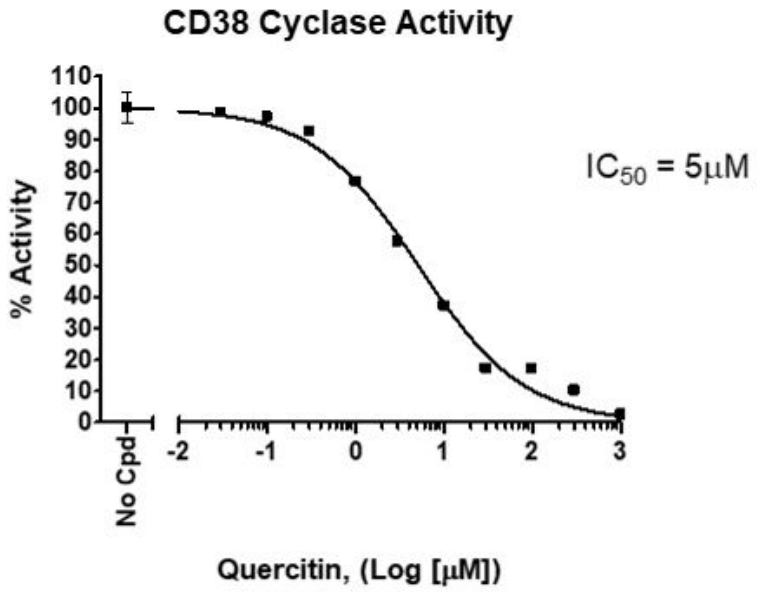
8. During incubation, dilute **NGD⁺** 10-fold with **1x CD38 assay buffer**. Dilute only the amount required for the assay. Store remaining **NGD⁺** at -20°C in single use aliquots. Discard any unused diluted **NGD⁺** after use.

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 quercitin, measured using the ***CD38 Inhibitor Screening Assay Kit (cyclase activity)***, BPS Bioscience Cat. # 71275. Fliuorescence 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:

Product Name	Catalog #	Size
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