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Data Sheet

CD38 Inhibitor Screening Assay Kit (Hydrolase Activity)

Catalog # 79287

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. The major enzymatic activity of CD38 is the hydrolysis of NAD. CD38 is a prognostic biomarker for acute B lymphoblastic leukemia.

DESCRIPTION: The *CD38 Inhibitor Screening Assay Kit (Hydrolase Activity)* is designed to measure the glycohydrolase 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 N6-etheno-NAD (ε-NAD), and CD38 assay buffer for 100 enzyme reactions. In addition, the kit includes the CD38 inhibitor apigenin for use as a control inhibitor.

COMPONENTS:

Catalog #	Reagent	Amount	Storage	
71277	CD38, His-Tag (Human), HiP™	1 µg	-80°C	***Avoid multiple freeze/thaw cycles!***
	4x CD38 hydrolase buffer	3 ml	-20°C	
	CD38 substrate (ε-NAD)	50 µl	-20°C	
	Apigenin (50 mM DMSO)	10 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 **4x CD38 hydrolase buffer** on ice.

2. Prepare the master mixture (10 µl per well): N wells x (5 µl **4x CD38 hydrolase buffer** + 5 µl water). Add 10 µl to every well.

	Positive Control	Negative Control*	Test Inhibitor	Blank
4x CD38 hydrolase buffer	5 µl	5 µl	5 µl	5 µl
Water	5 µl	5 µl	5 µl	5 µl
Test Inhibitor	–	–	10 µl	–
Inhibitor Buffer (no inhibitor)	10 µl		–	10 µl
Apigenin	–	10 µl	–	–
1x CD38 hydrolase buffer	–	–	–	20 µl
CD38 (0.5 ng/µl)	20 µl	20 µl	20 µl	–
ε-NAD (diluted)	10 µl	10 µl	10 µl	10 µl
Total	50 µl	50 µl	50 µl	50 µl

3. Prepare **1x CD38 hydrolase buffer** by diluting **4x CD38 hydrolase buffer** with water. Dilute only enough buffer required for the assay. Store remaining **4x CD38 hydrolase buffer** at -20°C in single-use aliquots. For 100 reactions, prepare 6 ml **1x CD38 hydrolase buffer** by mixing 1.5 ml of **4x CD38 hydrolase buffer** with 4.5 ml water.

4. 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).
*Optional: For the well labeled “Negative Control”, add 10 µl apigenin, diluted 0.1 – 100 µM in 1x CD38 hydrolase buffer.

5. To the wells designated as "Blank", add 20 µl of **1x CD38 hydrolase 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 0.5 ng/µl with **1x CD38 assay buffer** (10 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.*

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7. Add 20 µl of diluted **CD38** enzyme to the wells designated “Positive Control”, “Negative Control”, and "Test Inhibitor Control". Cover the plate and incubate 30 minutes at room temperature with slow shaking.

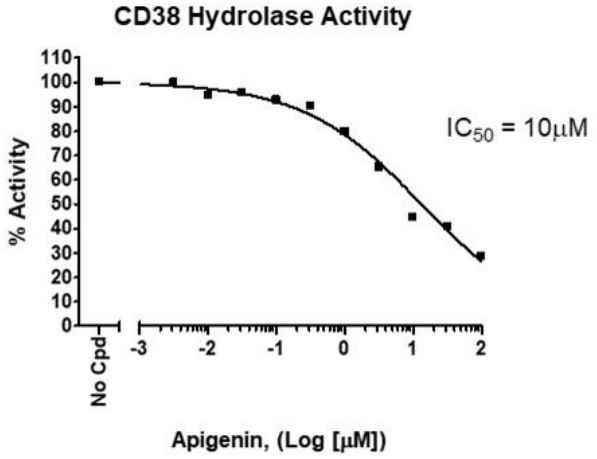
8. During incubation, dilute **ε-NAD** 20-fold with **1x CD38 hydrolase buffer**. Dilute only the amount required for the assay. Store remaining **ε-NAD** at -20°C in single use aliquots. Discard any unused diluted **ε-NAD** after use.

9. After the 30 minute incubation, remove the plate and add 10 µl of diluted **ε-NAD**.

10. Place plate into plate-reading fluorimeter and prepare to measure.

11. After 10-12 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 apigenin, measured using the ***CD38 Inhibitor Screening Assay Kit (Hydrolase Activity)***, BPS Bioscience Cat. # 79287. 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:

Product Name	Catalog #	Size
CD38, His-Tag (Human), HiP™	71227	100 µg
CD39, His-tag	71284	20 µg
CD73, Avi, His-tag (Mouse)	72523	100 µg
CD73, His-tag	71184	50 µg
CD73 Inhibitor Screening Assay Kit	72055	96 rxns
CD73 Inhibitor Screening Assay Kit	72058	384 rxns
CD38 Inhibitor Screening Assay Kit	71275	96 rxns.
CD39 Inhibitor Screening Assay Kit	79278	96 rxns.
Quercetin	27214	5 g
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