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Data sheet
Rat CD38 Inhibitor Screening Assay Kit (Hydrolase Activity)
Catalog #79690
Size: 96 reactions

BACKGROUND: CD38, a differentiation antigen of B lymphocytes, is a type II integral membrane protein that functions as an ADP-ribosyl cyclase and nicotinamide adenine dinucleotide (NAD) glycohydrolase. The major enzymatic activity of CD38 is the hydrolysis of NAD. 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 *Rat CD38 Inhibitor Screening Assay Kit (Hydrolase Activity)* is designed to measure the glycohydrolase activity of rat CD38 for screening and profiling applications. The CD38 assay kit comes in a convenient 96-well format, with recombinant rat CD38 enzyme, its substrate N6-etheno-NAD (ϵ -NAD), and CD38 assay buffer for 96 enzyme reactions. In addition, the kit includes the CD38 inhibitor apigenin for use as a control inhibitor.

COMPONENTS:

Catalog #	Component	Amount	Storage	
100269	CD38 (Rat)	1 μ g	-80°C	Avoid multiple freeze/thaw cycles!
	4x CD38 hydrolase buffer	4 ml	-20°C	
	CD38 substrate (ϵ -NAD)	50 μ l	-20°C	
	Apigenin (50 mM in DMSO)	10 μ l	-20°C	
79685	Black 96-well plate			

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.

REFERENCES:

Koguma, T., *et al.* *Biochim. Biophys. Acta Mol. Cell. Res.* 1994. **1223**(1): 160-162
Malavasi, F., *et al.* *Physiol. Rev.* 2008. **88**(3): 841-886

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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 μ l per well): N wells x (5 μ l **4x CD38 hydrolase buffer** + 5 μ l distilled water). Add 10 μ l to every well.

	Positive Control	Negative Control	Test Inhibitor	Blank
4x CD38 hydrolase buffer	5 μ l	5 μ l	5 μ l	5 μ l
Distilled water	5 μ l	5 μ l	5 μ l	5 μ l
Test Inhibitor	-	-	10 μ l	
Inhibitor buffer (no inhibitor)	10 μ l	-	-	10 μ l
Apigenin	-	10 μ l	-	-
1x CD38 hydrolase buffer	-	-	-	20 μ l
Rat CD38 (0.25 ng/ μ l)	20 μ l	20 μ l	20 μ l	-
ϵ -NAD (diluted)	10 μ l	10 μ l	10 μ l	10 μ l
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 96 reactions, prepare 10 ml **1x CD38 hydrolase buffer** by mixing 2.5 ml of **4x CD38 hydrolase buffer** with 7.5 ml water.
4. 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). For the wells labeled "Negative Control", add 10 μ l apigenin, diluted 0.1 – 100 μ M in 1x CD38 hydrolase buffer.
5. To the wells designated as "Blank", add 20 μ l of **1x CD38 hydrolase buffer**.
6. Thaw **rat CD38** enzyme on ice. Upon first thaw, briefly spin tube containing enzyme to recover full contents of the tube. Calculate the amount of **rat CD38** required for the assay and dilute enzyme to 0.25 ng/ μ l with **1x CD38 assay buffer** (5 ng/well). Aliquot remaining **rat CD38** enzyme into single-use aliquots. Store remaining undiluted enzyme in aliquots at -80°C. *Note:* **rat 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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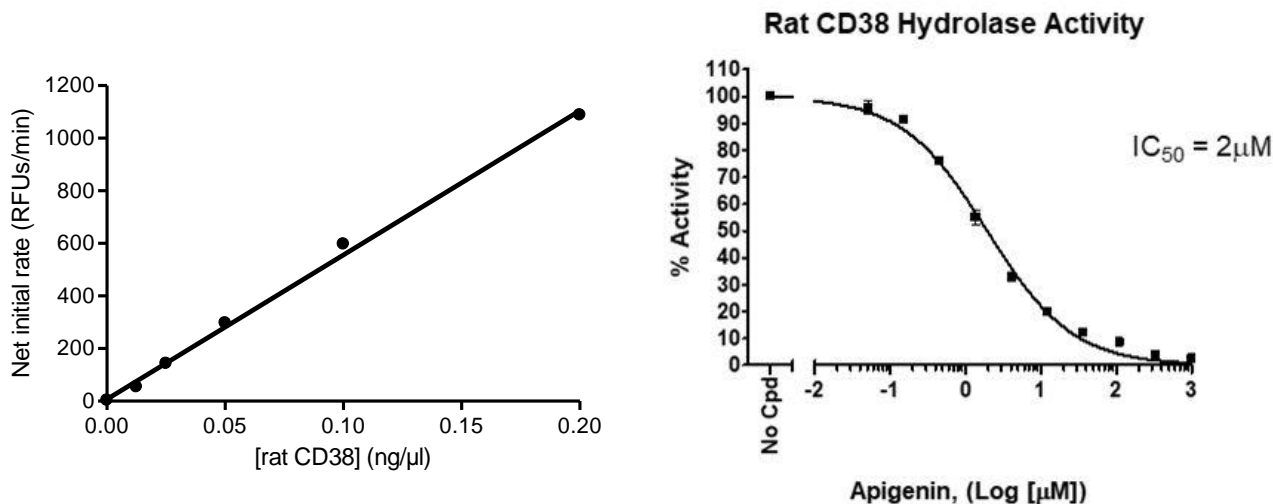
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7. Add 20 μ l of diluted **rat CD38** enzyme to the wells designated "Positive Control", "Negative Control", and "Test Inhibitor Control". Cover the plate and incubate 30 minutes at room temperature with slow shaking.
8. During incubation, dilute **ϵ -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 minutes incubation, remove the plate and add 10 μ l of diluted **ϵ -NAD**.
10. Place plate into plate-reading fluorimeter and prepare to measure.
11. After 10 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 Assay Results:



Rat CD38 activity (left) and inhibition by apigenin (right) measured using the **Rat CD38 Inhibitor Screening Assay Kit (Hydrolase Activity)**, BPS Bioscience #79690. 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™	71277	100 µg
CD38, His-Tag (Mouse), HiP™	79070	100 µg
CD38 Inhibitor Screening Assay Kit (Hydrolase Activity)	79287	96 rxns
CD38 Inhibitor Screening Assay Kit (Hydrolase Activity)	79672	384 rxns
CD38 Inhibitor Screening Assay Kit (Cyclase Activity)	71275	96 rxns
Mouse CD38 Inhibitor Screening Assay Kit (Hydrolase Activity)	79682	96 rxba
CD39, His-tag	71284	20 µg
CD39 Inhibitor Screening Assay Kit	79278	96 rxns
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
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

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