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Produktinformation

- Forschungsprodukte & Biochemikalien
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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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CD38 (Mouse) Fluorogenic Assay Kit (Cyclase Activity)

#78285
96 reactions

Description

The CD38 (Mouse) Inhibitor Screening Assay Kit (Cyclase Activity) is designed to measure the cyclase activity of CD38 (mouse) 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 an inhibitor control.

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, where it regulates insulin secretion. CD38 is a prognostic biomarker for acute B lymphoblastic leukemia, and anti-CD38 antibodies are in clinical trials as therapeutics for multiple myeloma.

Applications

Useful for studying enzyme kinetics and screening small molecular inhibitors for drug discovery and HTS applications.

Supplied Materials

Catalog #	Name	Amount	Storage
79070	CD38, His-Tag (Mouse), HiP™	2 x 1 µg	-80°C
	3x CD38 cyclase assay buffer	4 ml	-20°C
	CD38 substrate NGD^+	50 µl	-20°C
	Quercetin (50 mM in DMSO)	100 µl	-20°C
79685	Black 96-well plate	1	Room Temp.

The initial concentration of CD38 is lot-specific and will be indicated on the tube containing the protein.

Materials Required but Not Supplied

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

Storage Conditions

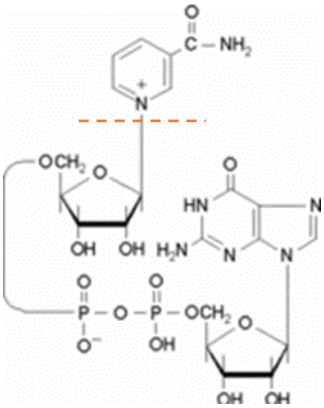
This assay kit will perform optimally for up to 6 months from date of receipt when the materials are stored as directed.

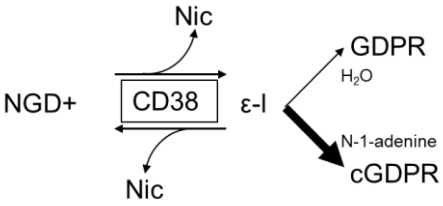
Safety

This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

Assay Principle

Cyclase kits use NGD+ (Nicotinamide Guanine Dinucleotide). The cyclase reaction preferentially generates a cyclic GDP-ribose product, which is fluorescent (Ex/Em = 300nm/410nm), while the hydrolyzed product is not fluorescent.





Contraindications

The final concentration of DMSO in the reaction should be ≤1%.

Assay Protocol

All samples and controls should be tested in duplicate. We recommend preincubating the enzyme with the inhibitor. However, it is acceptable to add the substrate mixture and inhibitor followed by diluted murine CD38 (mCD38) without the preincubation step.

Preparing Your Reagents

1. Thaw 3x CD38 assay buffer on ice.
2. Add 10 µl of 3x CD38 assay buffer and 10 µl of distilled water to each well
3. Prepare 1x CD38 assay buffer by diluting 3x CD38 assay buffer with distilled water. Dilute only enough buffer required for the assay. Store the remaining 3x CD38 assay buffer at -20°C in single-use aliquots. For 100 reactions, prepare 6 ml of 1x CD38 assay buffer by mixing 2 ml of 3x CD38 assay buffer with 4 ml of water.
4. Prepare the test compound by making a 100x solution. Dilute 1:20 with 1x CD38 assay buffer to make a 5x solution. Note that if the compound is dissolved in 100% DMSO, this brings DMSO to 5%. Add 10 µl of diluted test compound to each well labeled as “Test Inhibitor”. For the wells labeled “Positive Control" and “Blank”, add 10 µl of the diluent solution (5% DMSO in 1x CD38 assay buffer without the test compound, if diluent is DMSO).
5. To the wells designated as "Blank", add 15 µl of 1x CD38 assay buffer.
6. Thaw mouse CD38 enzyme on ice. Briefly spin tube containing enzyme to recover full contents of the tube. Calculate the amount of mCD38 required for the assay and dilute enzyme to 1.33 ng/µl with 1x CD38 assay buffer (20 ng/well). Aliquot remaining undiluted mCD38 enzyme into single-use aliquots and store remaining at -80°C.
   Note: mouse 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 mCD38 enzyme to the wells designated “Positive Control” and "Test Inhibitor Control" and 15 µl of 1x CD38 assay buffer to “Blank”.
8. Cover the plate and incubate 1 hour at room temperature with slow shaking.

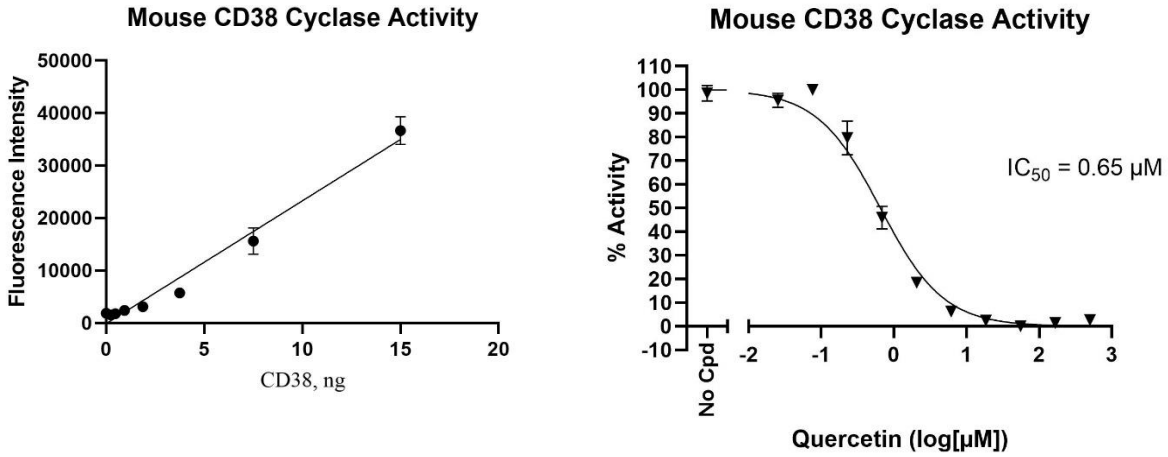
Component	Blank	Positive Control	Test Inhibitor
3x CD38 assay buffer	10 µl	10 µl	10 µl
Distilled water	10 µl	10 µl	10 µl
Test Inhibitor			10 µl
Inhibitor buffer (5% DMSO in 1x buffer)	10 µl	10 µl	
1x CD38 assay buffer	15 µl		
Mouse CD38 (1.33 ng/µl)		15 µl	15 µl
Total	45 µl	45 µl	45 µl

9. During the incubation, dilute NGD^+ 10-fold with 1x CD38 assay buffer. Dilute only the amount required for the assay. Store remaining undiluted NGD^+ at -20°C in single use aliquots. Discard any unused diluted NGD^+ after use.

Initiating the reaction

10. After the 1-hour incubation, **add 5 µl of diluted NGD^+ to every well.** This brings the final volume to 50 µl.
11. Incubate for 10 minutes at room temperature.
12. After the 10-minute incubation, 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 Results



Mouse CD38 cyclase activity using increasing amounts of enzyme (left) and inhibition of CD38 by increasing concentrations of quercetin (right) measured using the CD38 (mouse) Inhibitor Screening Assay Kit (Cyclase

Activity), BPS Bioscience #78285 Fluorescence was measured using a Tecan microplate reader. Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com

General Considerations

"Blank" Control: The "Blank" control is important to determine the background luminescence in the assay. We recommend doing these in duplicate.

"Positive Control":

The "Positive Control" is the maximum signal determined upon the addition of diluent solution (for example 1% DMSO in 1x CD38 Assay Buffer) in the absence of inhibitor.

Troubleshooting Guide

Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com

Reference

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

Related Products

Products	*Catalog #*	*Size*
CD38, FLAG-Tag (Pig), HiP™	101019	100 µg
CD38, Avi-His-Tag (Human)	100346	100 µg
CD38, His-Tag (Mouse), HiP™	79070	100 µg
CD38, His-Tag (Dog)	100955	100 µg
CD38 (Pig) Fluorogenic Assay Kit (Hydrolase Activity)	78178	96 reactions
CD38 (Dog) Inhibitor Screening Assay Kit (Hydrolase Activity)	78108	96 reactions
CD38 (Rat) Inhibitor Screening Assay Kit (Hydrolase Activity)	79690	96 reactions
CD38 (Mouse) Inhibitor Screening Assay Kit (Hydrolase Activity)	79287/79682	96 reactions/384 reactions
CD38 Inhibitor Screening Assay Kit (Cyclase Activity)	71275	96 reactions
CD38 / BCMA / Firefly Luciferase CHO Recombinant Cell Line	78148	2 vials
CD38 / CD19 / Firefly Luciferase CHO Recombinant Cell Line	78149	2 vials
CD38 CHO Recombinant Cell Line (High, Medium or Low Expression)	79615	2 vials