

Description

The Pig CD38 Inhibitor Screening Assay Kit (Hydrolase Activity) is designed to measure the glycohydrolase activity of porcine CD38 for screening and profiling applications. The CD38 assay kit comes in a convenient 96-well format, with recombinant pig 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.

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 main 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.

Applications

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

Supplied Materials

| Catalog # | Name | Amount | Storage |
|-----------|-----------------------------------|-------------|-----------|
| 101019 | CD38 (Pig) | 25 μ g | -80°C |
| | 4x CD38 hydrolase buffer | 4 ml | -20°C |
| | CD38 substrate (ϵ -NAD) | 200 μ l | -20°C |
| | Apigenin (50 mM in DMSO) | 10 μ l | -20°C |
| 79685 | Black 96-well plate | 1 | Room Temp |
| | Plate sealing film | 2 | Room Temp |

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 Protocol

All samples and controls should be tested in duplicate. We recommend preincubating the enzyme with inhibitor.

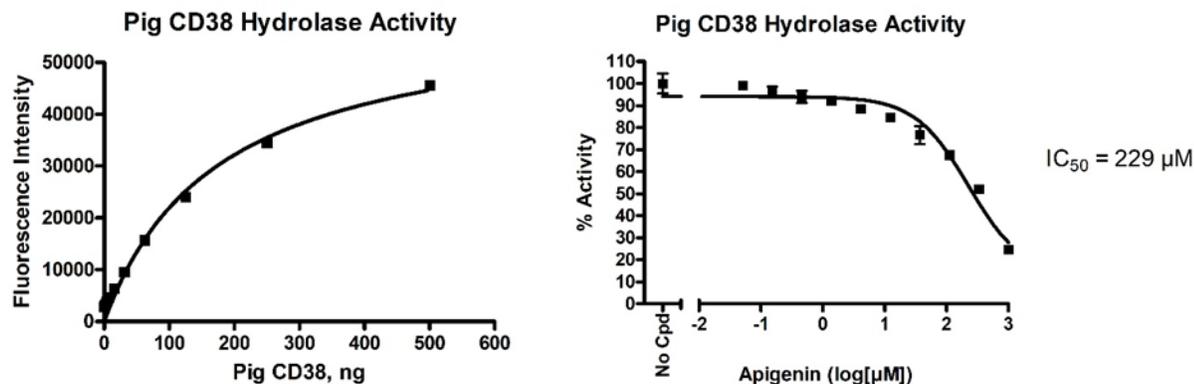
1. Thaw **4x CD38 hydrolase buffer** on ice.
2. Prepare the master mixture (10 μ l per well): N wells x (5 μ l **4x CD38 hydrolase buffer** + 5 μ l distilled water). Add 10 μ l to every well.

| Component | Blank | Negative Control | Positive Control | Test Inhibitor |
|---------------------------------|------------|------------------|------------------|----------------|
| 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 |
| Pig CD38 (12.5 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 μ l | 50 μ l | 50 μ l | 50 μ l |

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 to 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 **pig CD38** enzyme on ice. Upon first thaw, briefly spin tube containing enzyme to recover full contents of the tube. Calculate the amount of **pig CD38** required for the assay and dilute enzyme to 12.5 ng/ μ l with **1x CD38 assay buffer** (250 ng/well). Aliquot remaining **pig CD38** enzyme into single-use aliquots. Store remaining undiluted enzyme in aliquots at -80°C. *Note:* **pig CD38** enzyme is sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.
7. Add 20 μ l of diluted **pig CD38** enzyme to the wells designated "Positive Control", "Negative Control", and "Test Inhibitor Control". Omit the enzyme in wells designated "Blank". Cover the plate and incubate 30 minutes at room temperature with slow shaking.
8. During incubation, dilute **ϵ -NAD** 5-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 pre-incubation, remove the plate and add 10 μ l of diluted ϵ -NAD to each well.
10. Seal plate and incubate overnight with slow shaking.
11. After overnight 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



Pig CD38 activity (left) and inhibition by apigenin (right) measured using the **Pig CD38 Inhibitor Screening Assay Kit (Hydrolase Activity)**, BPS Bioscience #78178. Fluorescence was measured using a Bio-Tek microplate reader. Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com

General Considerations

Plates and Instruments: A plate reader capable of Alpha technology detection is required. We recommend using PerkinElmer 384-Optiplate #6007290.

“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 by the addition of a system with DMSO, in our case the “10% DMSO in water (Inhibitor buffer).”

Trouble Shooting Guide

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

Related Products

| <i>Products</i> | <i>Catalog #</i> | <i>Size</i> |
|---|------------------|---------------|
| CD38 (Pig) | 101019 | xxx µg |
| 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 reactions |
| CD38 Inhibitor Screening Assay Kit (Hydrolase Activity) | 79672 | 384 reactions |
| CD38 Inhibitor Screening Assay Kit (Cyclase Activity) | 71275 | 96 reactions |
| Mouse CD38 Inhibitor Screening Assay Kit (Hydrolase Activity) | 79682 | 96 reactions |
| CD39, His-tag | 71284 | 20 µg |
| CD39 Inhibitor Screening Assay Kit | 79278 | 96 reactions |
| CD73, Avi, His-tag (Mouse) | 72523 | 100 µg |
| CD73, His-tag | 71184 | 50 µg |
| CD73 Inhibitor Screening Assay Kit | 72055 | 96 reactions |
| CD73 Inhibitor Screening Assay Kit | 72058 | 384 reactions |
| 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), GST-tag | 91004 | 50 µg |
| NMNAT, His-tag | 71090 | 100 µg |
| TCF/LEF Reporter Kit | 60500 | 500 reactions |
| TCF/LEF reporter-HEK293 Cell Line | 60501 | 2 vials |