SZABO SCANDIC

Part of Europa Biosite

Produktinformation

- Forschungsprodukte & Biochemikalien
- Zellkultur & Verbrauchsmaterial
- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

Weitere Information auf den folgenden Seiten!
See the following pages for more information!

Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

linkedin.com/company/szaboscandic

Description

The CD200R1:CD200 [Biotinylated] Inhibitor Screening Chemiluminescence Assay Kit is an ELISA designed to assess the efficacy of inhibitors of the binding between CD200R1 (CD200 receptor 1) and CD200 (cluster of differentiation 200) protein for screening and profiling applications. This kit comes with enough purified CD200R1 (amino acids 29-265) and biotinylated CD200 (amino acids 31-232), streptavidin-HRP, assay buffer, and detection reagent for 100 enzyme reactions.

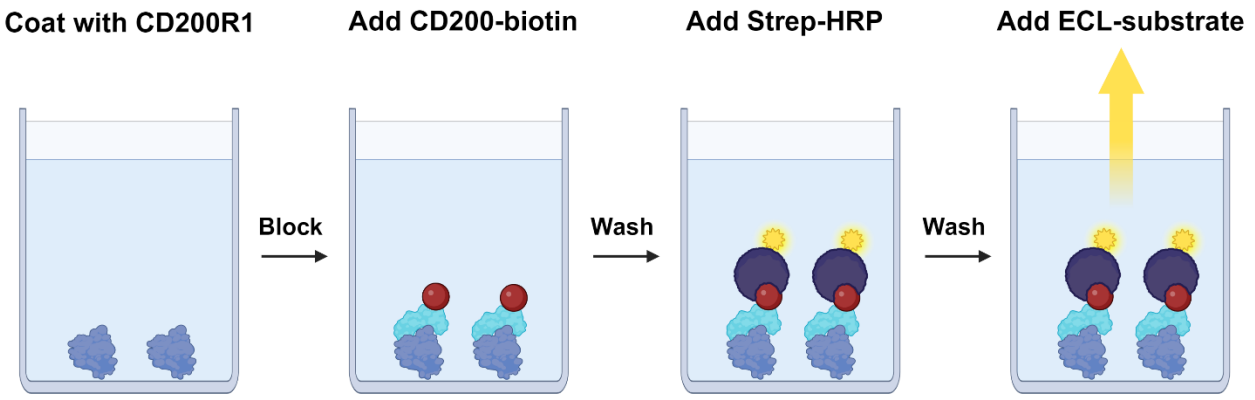

Figure 1. CD200R1:CD200 [Biotinylated] Inhibitor Screening Chemiluminescence Assay Kit schematic.
A 96-well plate is coated with CD200R1 protein. After blocking, biotinylated CD200 is added in an optimized assay buffer. Next, unbound biotinylated CD200 is washed away, and the plate is incubated with streptavidin-HRP. After a final wash, ELISA ECL substrate is added to produce chemiluminescence that can be measured using a chemiluminescence reader. The chemiluminescence signal is proportional to CD200 binding to CD200R1.

Background

CD200 (Cluster of Differentiation 200) and its receptor, CD200R, are cell surface proteins that play a crucial role in immune regulation and tolerance. The CD200-CD200R pathway is primarily involved in inhibiting immune responses, helping to maintain immune homeostasis and prevent excessive inflammation. In many cancers, tumor cells overexpress CD200, which interacts with CD200R on immune cells, particularly myeloid cells like macrophages and dendritic cells. This interaction leads to the suppression of immune responses against the tumor, effectively promoting immune evasion. Neutralizing antibodies against CD200 or CD200R can block this interaction, preventing the inhibitory signal from being delivered to immune cells. As a result, the anti-tumor immune response is enhanced, leading to improved recognition and elimination of tumor cells by the immune system. The development of strategies targeting this complex holds great potential in cancer therapy.

Applications

Study and screen compounds that inhibit the binding of CD200R1 to CD200 for drug discovery in high throughput screening (HTS) applications.

Supplied Materials

Catalog #	Name	Amount	Storage
102034	CD200R1, His-Tag*	5 µg	-80°C
102014	CD200, Fc Fusion, Avi-Tag, Biotin-Labeled Recombinant*	> 0.5 µg	-80°C
82620	5x PP-02 Buffer	4 ml	-20°C
79743	Blocking Buffer 3	50 ml	+4°C
79742	Streptavidin HRP	10 µl	+4°C C
79670	ELISA ECL Substrate A (translucent bottle)	6 ml	Room Temp
	ELISA ECL Substrate B (brown bottle)	6 ml	Room Temp
79699	White 96-well microplate	1	Room Temp

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

Materials Required but Not Supplied

- 1x PBS (Phosphate Buffer Saline) Buffer
- PBST Buffer (1x PBS, containing 0.05% Tween-20)
- Luminometer or microplate reader capable of reading chemiluminescence
- Adjustable micropipettor and sterile tips
- 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.

Contraindications

The CD200R1:CD200 Binding Assay Kit is compatible with up to 1% final DMSO concentration.

Assay Protocol

- All samples and controls should be performed in duplicate.
- The assay should include “Blank”, “Positive Control”, and “Test Inhibitor” conditions.
- We recommend maintaining the diluted protein on ice during use.
- For detailed information on protein handling please refer to Protein FAQs (bpsbioscience.com).

- We recommend using Anti-CD200 Neutralizing Antibody (#102062) as internal control. If not running a dose response curve for the control inhibitor, we recommend running the control inhibitor at 0.1X, 1X and 10X the IC_{50} value shown in the validation data below.
- For instructions on how to prepare reagent dilutions please refer to Serial Dilution Protocol (bpsbioscience.com).

Step 1: Coat 96-well plate

Coat the plate one day prior to running your samples.

1. Thaw CD200R1 on ice. Briefly spin the tube containing the protein to recover its full content.
2. Dilute CD200R1 protein to 1 ng/µl with 1x PBS (50 µl/well).
3. Add 50 µl of diluted CD200R1 to every well, except "Blank" wells.
4. Add 100 µl of Blocking Buffer 3 to the "Blank" wells.
5. Incubate at 4°C overnight.
6. Wash the plate three times using 200 µl of PBST Buffer per well.
7. Tap the plate onto a clean paper towel to remove the liquid.
8. Block the wells by adding 200 µl of Blocking Buffer 3 to every well.
9. Incubate at Room Temperature (RT) for at least 90 minutes.
10. Wash the plate three times using 200 µl of PBST Buffer per well.
11. Tap the plate onto a clean paper towel to remove the liquid.

Step 2: Binding reaction

1. Prepare 1x Assay Buffer by diluting 5x PP-02 Assay Buffer 5-fold with distilled water.
2. Add 20 µl of 1x Assay Buffer to every well.
3. Prepare the Test Inhibitor/Blocker (5 µl/well): for a titration prepare serial dilutions at concentrations 10-fold higher than the desired final concentrations. The final volume of the reaction is 50 µl.

   3.1 If the Test Inhibitor/Blocker is soluble in water, prepare a solution of the compound that is 10-fold higher than the final desired concentration using 1x Assay Buffer.

   For the positive and negative controls, use 1x Assay Buffer (Diluent Solution).

OR

3.2 If the Test Inhibitor/Blocker is dissolved in DMSO, prepare a solution of the compound in 100% DMSO that is 100-fold higher than the highest concentration of the serial dilution. Then dilute 10-fold with 1x Assay Buffer (at this step the compound concentration is 10-fold higher than the desired final concentration). The concentration of DMSO in the dilution is now 10%.

Prepare serial dilutions of the Test Inhibitor at concentrations 10-fold higher than the desired final concentrations using 10% DMSO in 1x Assay Buffer to keep the concentration of DMSO constant.

For positive and negative controls, prepare 10% DMSO in 1x Assay Buffer (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

Note: The final concentration of DMSO should not exceed 1%.

4. Add 5 µl of Test Inhibitor to each well labeled as "Test Inhibitor".
5. Add 5 µl of Diluent Solution to the "Positive Control" and "Blank" wells.
6. Thaw CD200 on ice. Briefly spin the tube containing the protein to recover its full content.
7. Dilute CD200 to 0.2 ng/µl with 1x Assay Buffer (25 µl/well).
8. Add 25 µl of diluted CD200 to all wells.
9. Incubate at RT for 1 hour.

	Blank	**Positive Control**	**Test Inhibitor**
1x Assay Buffer	20 µl	20 µl	20 µl
Test Inhibitor	-	-	5 µl
Diluent Solution	5 µl	5 µl	-
Diluted CD200 (0.2 ng/µl)	25 µl	25 µl	25 µl
Total	**50 µl**	**50 µl**	**50 µl**

10. Wash the plate three times with 200 µl of PBST Buffer per well and tap the plate onto a clean paper towel.
11. Add 100 µl of Blocking Buffer 3 to all wells and incubate for 10 minutes.
12. Tap plate onto a clean paper towel.

Step 3: Detection

1. Dilute 1000-fold the Streptavidin-HRP with Blocking Buffer 3 (50 µl/well).
2. Add 50 µl of diluted Streptavidin-HRP to every well.
3. Incubate for 1 hour at RT.
4. Wash the plate three times with 200 µl of PBST Buffer per well and tap the plate onto clean paper towel.
5. Just before use, mix 1 volume of ELISA ECL Substrate A and 1 volume of ELISA ECL Substrate B (100 µl of mix/ well).
6. Add 100 µl of mix to every well.
7. Immediately read the plate in a luminometer or microtiter-plate reader capable of reading chemiluminescence.
8. The "Blank" value should be subtracted from all other values.

Reading Chemiluminescence

Chemiluminescence is the emission of light (luminescence) which results from a chemical reaction. The detection of chemiluminescence requires no wavelength selection because the method used is emission photometry and is not emission spectrophotometry.

To properly read chemiluminescence, make sure the plate reader is set for LUMINESCENCE mode. Typical integration time is 1 second, delay after plate movement is 100 msec. Do not use a filter when measuring light emission. Typical settings for the Synergy 2 BioTek plate reader are: use the "hole" position on the filter wheel; Optics position: Top; Read type: endpoint. Sensitivity may be adjusted based on the luminescence of controls.

Example Results

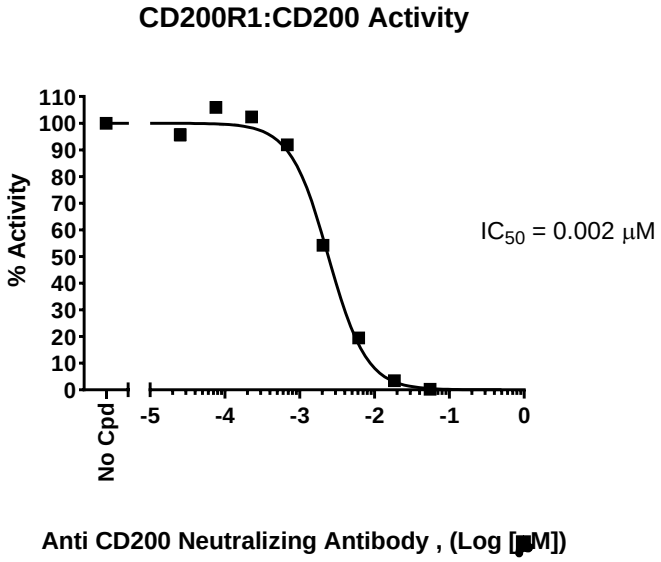

Figure 2: Inhibition of CD200R1 -CD200 binding by Anti-CD200 Neutralizing Antibody.
CD200 was incubated with increasing concentrations of Anti-CD200 Neutralizing Antibody (#102062) in a CD200R1 coated plate. Luminescence was measured using a Bio-Tek microplate reader. Results are expressed as a percentage of binding activity in which the condition without Anti-CD200 Neutralizing Antibody is set to 100%.

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.

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

Related Products

Products	*Catalog #*	*Size*
Anti-CD200 Neutralizing Antibody	102062	50 µg/100 µg
CD200 :CD200R1[Biotinylated] Inhibitor Screening Chemiluminescence Assay Kit	82285	96 reactions
CD200, Fc Fusion, Avi-Tag Recombinant	102013	100 µg
CD200, Fc Fusion, Avi-Tag, Biotin-Labeled Recombinant	102014	25 µg/100 µg
CD200R1, Fc Fusion, Avi-Tag Recombinant	102032	100 µg
CD200R1, Fc Fusion, Avi-Tag, Biotin-Labeled Recombinant	102033	25 µg/100 µg

Version 012125