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 Fc (IgG1): FcRn Inhibitor Screening Chemiluminescent Assay Kit is designed for the screening and profiling of neutralizing antibodies or inhibitors of the interaction between human Fc (IgG1) and human FcRn (Neonatal Fc receptor for IgG). This kit comes in a convenient 384-well format, with purified Biotinylated-FcRn complex (Fc receptor amino acids 24-297 and B2M amino acids 21-119) and Fc (IgG1) (amino acids 100-330) proteins, Streptavidin-HRP, and assay buffers for 400 reactions.

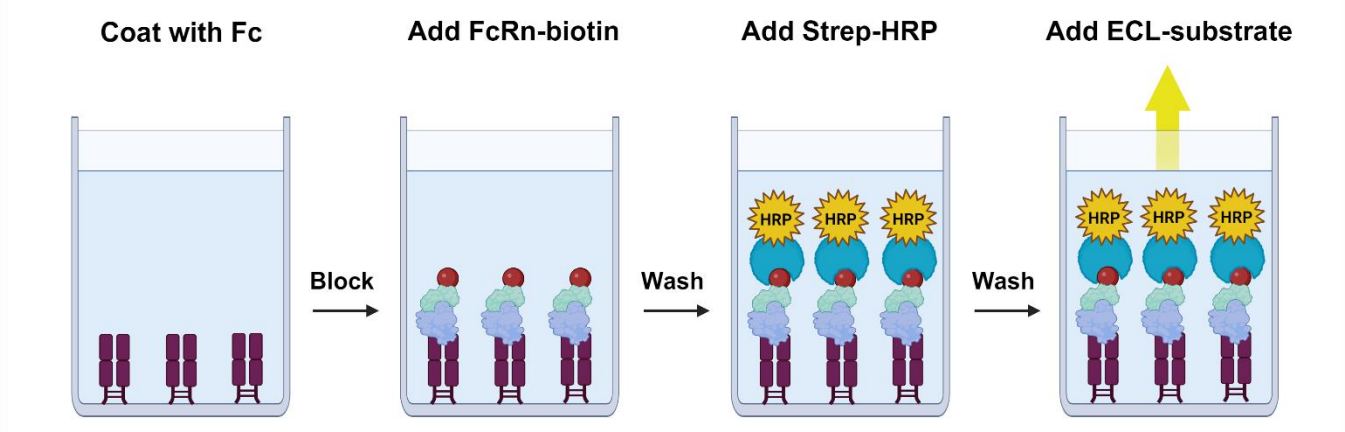

Figure 1: Illustration of the mechanism of Fc (IgG1): FcRn Inhibitor Screening Chemiluminescent Assay Kit. A 384-well plate is coated with Fc (IgG1) protein. After blocking, the plate is pre-incubated with an inhibitor or neutralizing antibody. Upon subsequent incubation with Biotin-FcRn, the plate is treated with Streptavidin-HRP followed by addition of the ELISA ECL substrate to produce chemiluminescence that can be measured using a chemiluminescence reader. The chemiluminescence signal is proportional to the binding of FcRn to Fc (IgG1).

Background

Neonatal Fc receptor for IgG (FcRn) is a heterodimeric protein. FcRn consists of the Fc Gamma Receptor and Transporter encoded by the FCGRT gene, associated with beta-2-Microglobulin (B2M). FcRn binds to the Fc region of monomeric immunoglobulin G (IgG). It is expressed in over 25 tissue types, with high expression levels observed in the spleen and intestine. In the placenta, it transports IgGs from mother to fetus. FcRn contributes to an effective humoral immunity by protecting IgGs from degradation, recycling them and extending their half-life in circulation. In addition to IgGs, it regulates the homeostasis of serum albumin. FcRn is a potential therapeutic target for autoimmune diseases. Disrupting the FcRn/IgG interaction is expected to increase the overall clearance of IgGs, including disease-causing autoantibodies. Engineered Fc fragments or neutralizing IgGs that bind to FcRn with high affinity through their Fc region are currently undergoing clinical trial. The first FDA-approved drug targeting FcRn (efgartigimod) is now used to treat myasthenia gravis, an autoimmune neuromuscular disease caused by the presence of autoantibodies against acetylcholine receptor, providing proof-of-concept in favor of this strategy.

Application(s)

Screen or titrate inhibitors of FcRn binding to Fc (IgG1).

Supplied Materials

Catalog #	Name	Amount	Storage
71456	IgG1, Fc (Human)*	2 x 10 µg	-80°C
71283	FcRn Complex (FCGRT/B2M), His-Avi-Tag, Biotin-Labeled*	2 x 5 µg	-80°C
82646	3x Acidic FcRn Wash Buffer	2 x 50 ml	-20°C
82609	5x FcRn Binding Buffer 2	2 x 1.5 ml	-20°C
78502	Blocking Buffer 6	2 x 50 ml	+4°C
79742	Streptavidin-HRP	2 x 10 µl	+4°C
79670	ELISA ECL Substrates A	2 x 6 ml	Room Temp
	ELISA ECL Substrates B	2 x 6 ml	Room Temp
78188	White 384-well microplate	1	Room Temp

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

Materials Required but Not Supplied

- PBS (Phosphate Buffered Saline)
- Luminometer or microplate reader capable of reading chemiluminescence
- Adjustable micropipettor and sterile tips
- Orbital shaker

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

Assay Protocol

- All samples and controls should be tested in duplicate.
- The assay should include “Non-Coated Control”, “Blank”, “Positive Control” and “Test Compound” wells.
- We recommend preincubating antibodies or protein inhibitors with the target protein prior to the addition of the binding partner.
- For small molecule inhibitors, pre-incubation may also be beneficial, depending on the experimental conditions.

- We recommend using FcRn (FCGRT/B2M) Blocker (#101468) as an internal control for the assay. If not running a dose response curve for the control inhibitor, run at 0.1X, 1X and 10X the IC_{50} value shown in the validation data below.
- We recommend maintaining the diluted protein on ice during use.
- For detailed information on protein handling please refer to Protein FAQs (bpsbioscience.com).
- For instructions on how to prepare reagent dilutions please refer to Serial Dilution Protocol (bpsbioscience.com).

Step 1 - Plate coating with Fc (IgG1) protein

1. Coat the plate one day prior to running your samples in the assay.
2. Thaw **Fc (IgG1)** protein on ice. Briefly spin the tube to recover the full content.
3. Dilute **Fc (IgG1)** protein to 2 ng/µl in PBS (25 µl/well).
4. Add 25 µl of diluted Fc (IgG1) protein solution to each well, except the "Non-Coated Control" wells.
5. Add 25 µl of PBS to the "Non-Coated Control" wells.
6. Incubate at 4°C overnight.
7. Prepare **1x Acidic FcRn Wash Buffer** by diluting 3-fold **3x Acidic FcRn Wash Buffer** with distilled water.
8. Tap the plate onto a clean paper towel to remove the liquid.
9. Wash the plate three times with 50 µl/well of 1x Acidic FcRn Wash Buffer.
10. Tap the plate onto a clean paper towel to remove the liquid.
11. Add 50 µl of Blocking Buffer 6 to every well.
12. Incubate for 1 hour at Room Temperature (RT) with gentle agitation.
13. Tap the plate onto a clean paper towel to remove the liquid.
14. Wash the plate three times with 50 µl/well of 1x Acidic FcRn Wash Buffer.
15. Tap the plate onto a clean paper towel to remove the liquid.
16. Start your assay test immediately.

Step 2: Reaction

1. Prepare **1x FcRn Binding Buffer 2** by diluting 5-fold the **5x FcRn Binding Buffer 2** with distilled water.

2. Add 10 µl of 1x FcRn Binding Buffer 2 to the "Non-Coated Control", "Positive Control" and "Test Compound" wells.

3. Add 22.5 µl of 1x FcRn Binding Buffer 2 to the "Blank" wells.

4. Prepare the Test Compound (2.5 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 25 µl.

   4.1 If the Test Compound is water-soluble, prepare serial dilutions in 1x FcRn Binding Buffer 2 at concentrations 10-fold higher than the desired final concentrations.

   OR

   4.2 If the Test Compound is soluble in DMSO, prepare the test inhibitor in 100% DMSO at a concentration 100-fold higher than the highest desired final concentration, then dilute the inhibitor 10-fold in 1x FcRn Binding Buffer 2 to prepare the highest concentration of the serial dilutions. The concentration of DMSO is now 10%.

   Using 1x FcRn Binding Buffer 2 containing 10% DMSO to keep the concentration of DMSO constant, prepare serial dilutions of the Test Inhibitor at 10-fold the desired final concentrations.

   For positive and negative controls, prepare 10% DMSO in 1x FcRn Binding Buffer 2 (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

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

5. Add 2.5 µl of Test Compound to each well designated "Test Compound".

6. Add 2.5 µl of Diluent Solution to the "Blank", "Non-Coated Control" and "Positive Control" wells.

7. Incubate at RT for 30 minutes with gentle agitation.

8. Thaw **FcRn Complex-biotin** on ice. Briefly spin the tube to recover its full content.

9. Dilute FcRn Complex-biotin to 2 ng/µl with 1x FcRn Binding Buffer 2 (12.5 µl/well).

10. Add 12.5 µl of diluted enzyme to the "Non-Coated Control, "Positive Control," and "Test Compound" wells.

11. Incubate at RT for 1 hour with gentle agitation.

12. Wash the plate three times with 50 µl/well of 1x Acidic FcRn Wash Buffer.

13. Tap the plate onto clean paper towels to remove liquid.

14. Add 50 µl of Blocking Buffer 6 to each well.

15. Incubate for 10 minutes at RT.

16. Tap the plate onto clean paper towels to remove liquid.

Step 3: Detection

1. Dilute **Streptavidin-HRP** 1000-fold with Blocking Buffer 6 (25 µl/well).

2. Add 25 µl of the diluted Streptavidin-HRP to each well.

3. Incubate the plate for 30 minutes at RT with gentle agitation.

4. Tap the plate onto clean paper towels to remove liquid.

5. Wash the plate three times with 50 µl/well of 1x Acidic FcRn Wash Buffer.

6. Just before use, prepare a mix (50 µl/well): N wells x (25 µl ELISA ECL Substrate A and 25 µl ELISA ECL Substrate B).

7. Add 50 µl of mix to each well.

   Note: Discard any unused chemiluminescent mix after use.

8. Immediately read the plate in a luminometer or plate reader capable of reading chemiluminescence.

9. The "Blank" value should be subtracted from all readings.

	Blank	**Non-Coated Control**	**Positive Control**	**Test Compound**
1x FcRn Binding Buffer 2	22.5 µl	10 µl	10 µl	10 µl
Test Compound	-	-	-	2.5 µl
Diluent Solution	2.5 µl	2.5 µl	2.5 µl	-
Pre-incubate 30 minutes at RT				
Diluted FcRn Complex-Biotin (2 ng/µl)	-	12.5 µl	12.5 µl	12.5 µl
Total	**25 µl**	**25 µl**	**25 µl**	**25 µl**

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 a control assay without enzyme (typically we set this value as 100).

Example Results

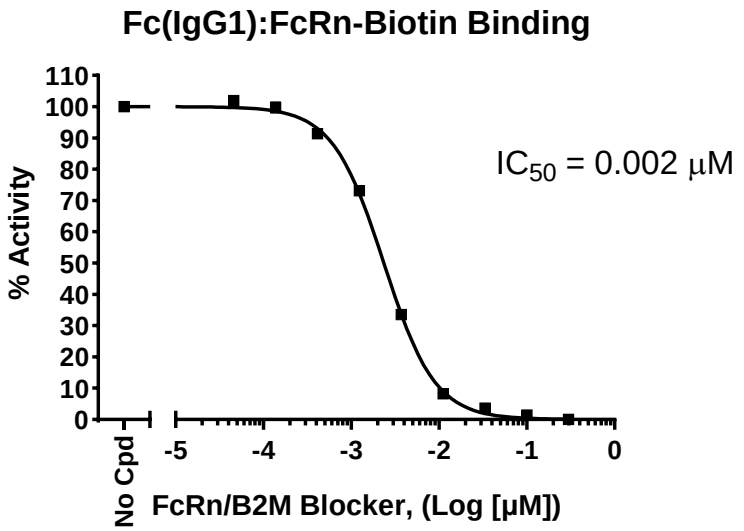

Figure 2. Inhibition of Fc (IgG1): FcRn binding by FcRn (FCGRT/B2M) Blocker.
Fc (IgG1): FcRn binding was evaluated in the presence of increasing concentrations of FcRn (FCGRT/B2M) Blocker (#101468). Results are expressed as percent activity, in which the binding activity in the absence of inhibitor is 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.

References
Dall'Acqua W.F., *et al.* 2002 *J Immunol.* 169(9): 5171-80.

Related Products

Products	*Catalog #*	*Size*
FcRn (FCGRT/B2M) Blocker	101468	100 µg
FcRn (FCGRT/B2M), His-Avi-Tag Recombinant	71285	100 µg/1 mg
FcRn (FCGRT/B2M), His-Tag (Mouse) HiP™ Recombinant	11349	25 µg/100 µg
FcRn (FCGRT/B2M), His-Avi-Tag, Biotin Labeled (Mouse) Recombinant	71286	50 µg
FcRn: IgG Recycling HMEC-1 Cell Pool	82163	2 vials

Version 090924