Description

The Fc (IgG1): FcRn Inhibitor Screening Colorimetric 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 96-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 100 reactions.

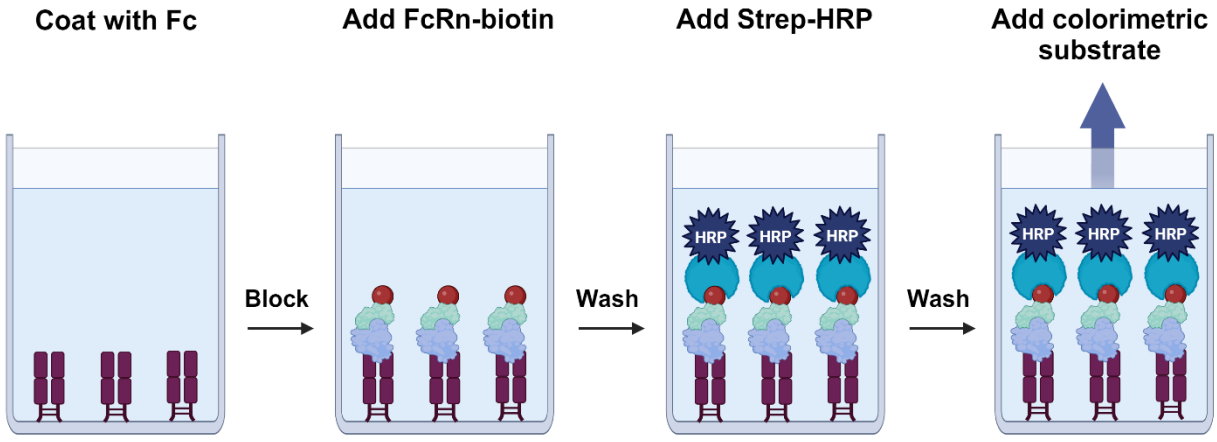

Figure 1: Illustration of the mechanism of Fc (IgG1): FcRn Inhibitor Screening Colorimetric Assay Kit. A 96-well plate is coated with Fc (IgG1) protein. After blocking, the plate is pre-incubated with an inhibitor or neutralizing antibody. Upon subsequent incubation with FcRn-biotin, the plate is treated with Streptavidin-HRP followed by addition of a colorimetric HRP substrate to produce color, which can be quenched and measured using a UV/Vis microplate reader. The signal is proportional to the binding of FcRn to Fc (IgG1).

Note: As of September 2024, this protocol has been re-optimized for performance.

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).

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Supplied Materials

Catalog #	Name	Amount	Storage
71456	IgG1, Fc (Human)*	10 µg	-80°C
71283	FcRn Complex (FCGRT/B2M), His-Avi-Tag, Biotin-Labeled*	5 µg	-80°C
82646	3x Acidic FcRn Wash Buffer	50 ml	-20°C
82609	5x FcRn Binding Buffer 2	1.5 ml	-20°C
78502	Blocking Buffer 6	50 ml	+4°C
79742	Streptavidin-HRP	10 µl	+4°C
79651	HRP Colorimetric Substrate	10 ml	+4°C
79964	Clear 96-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)
- 1N HCl (aqueous)
- Adjustable micropipettor and sterile tips
- Orbital shaker
- UV/Vis spectrophotometer microplate reader capable of reading absorbance at λ=450 nm

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%.
- Compounds that absorb/emit at 450 nm may interfere with the results, depending on their spectral excitation and emission properties.
- It is recommended that the compound alone is tested to determine any potential interference of the compound on the assay results.

Assay Protocol

- All samples should be run in duplicate while controls should be performed in quadruplicate.
- 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.

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- 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
Coat the plate one day prior to running your samples in the assay.

1. Thaw **Fc (IgG1)** protein on ice. Briefly spin the tube to recover the full content.
2. Dilute **Fc (IgG1)** protein to 2 ng/µl with PBS (50 µl/well).
3. Add 50 µl of diluted Fc (IgG1) protein solution to each well, except the "Non-Coated Control" wells.
4. Add 50 µl of PBS to the "Non-Coated Control" wells.
5. Incubate at 4°C overnight.
6. Prepare **1x Acidic FcRn Wash Buffer** by diluting 3-fold **3x Acidic FcRn Wash Buffer** with distilled water.
7. Tap the plate onto a clean paper towel to remove the liquid.
8. Wash the plate three times with 100 µl/well of 1x Acidic FcRn Wash Buffer.
9. Tap the plate onto a clean paper towel to remove the liquid.
10. Add 100 µl of Blocking Buffer 6 to every well.
11. Incubate for 1 hour at Room Temperature (RT) with gentle agitation.
12. Tap the plate onto a clean paper towel to remove the liquid.
13. Wash the plate three times with 100 µl/well of 1x Acidic FcRn Wash Buffer.
14. Tap the plate onto a clean paper towel to remove the liquid.
15. Start your assay immediately.

Step 2: Reaction

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

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2. Add 20 µl of 1x FcRn Binding Buffer 2 to the "Non-Coated Control", "Positive Control" and "Test Compound" wells.

3. Add 45 µl of 1x FcRn Binding Buffer 2 to the "Blank" wells.

4. Prepare the Test Compound (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.

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

6. Add 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 (25 µl/well).

10. Add 25 µl of diluted FcRn Complex to the "Non-Coated Control, "Positive Control," and "Test Compound" wells.

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

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	Blank	Non-Coated Control	Positive Control	Test Compound
1x FcRn Binding Buffer 2	45 µl	20 µl	20 µl	20 µl
Test Compound	-	-	-	5 µl
Diluent Solution	5 µl	5 µl	5 µl	-
Pre-incubate 30 minutes at RT				
Diluted FcRn Complex-Biotin (2 ng/µl)	-	25 µl	25 µl	25 µl
Total	**50 µl**	**50 µl**	**50 µl**	**50 µl**

12. Wash the plate three times with 100 µl/well of 1x Acidic FcRn Wash Buffer.
13. Tap the plate onto clean paper towels to remove liquid.
14. Add 100 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 (50 µl/well).
2. Add 50 µl of diluted Streptavidin-HRP to each well.
3. Incubate for 30 minutes at RT with gentle agitation.
4. Wash the plate three times with 100 µl/well of 1x FcRn Assay Wash Buffer.
5. Tap the plate onto clean paper towel to remove the liquid.
6. Prepare 1N HCl (aqueous) (100 µl/well). This is the **Stop Solution**.
7. Add 100 µl of **Colorimetric HRP Substrate** to each well.
8. Incubate the plate at RT until the "Positive Control" wells become blue.

   Note: This usually takes 1-5 minutes. The optimal incubation time may vary and should be determined empirically by the user.

9. Add 100 µl of Stop Solution to every well. The blue-colored solution will turn yellow.
10. Read the absorbance at λ=450 nm using an UV/Vis spectrophotometer microplate reader.

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Example Results

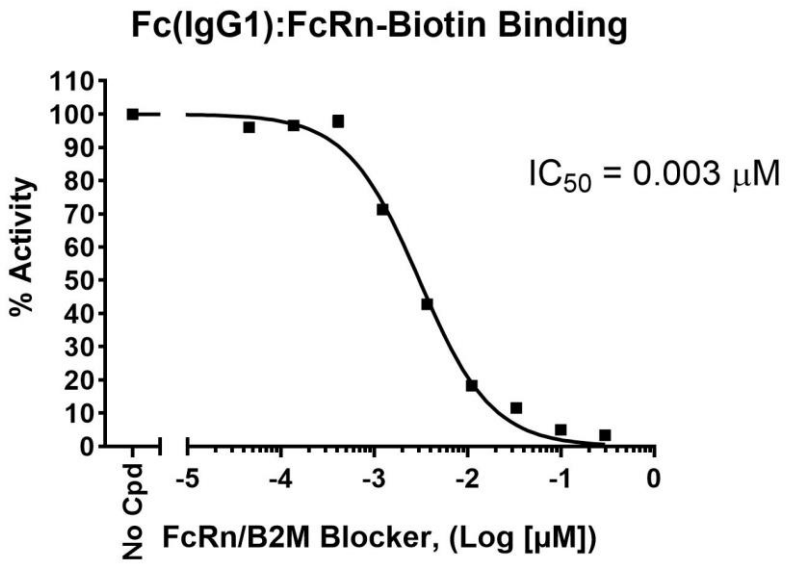

Figure 2. Inhibition of Fc (IgG1): FcRn binding by FcRn (FCGRT/B2M) Blocker.
Fc (IgG1): FcRn binding was evaluated in the presence of increasing concentrations of FcRn (FCGRT/B2M) Blocker (#101468). Results are expressed as percent activity, in which the binding activity in the absence of inhibitor is set to 100%.

Data shown is representative.

Troubleshooting Guide
Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For lot-specific information and all other questions, please visit https://bpsbioscience.com/contact.

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 052725

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