Description

The Fc (IgG1): FcRn Inhibitor Screening Colorimetric Assay Kit is designed for screening and profiling neutralizing antibodies or inhibitors of the interaction between Fc (IgG1) and human FcRn. This kit comes in a convenient 96-well format, with purified Biotinylated-FcRn and Fc (IgG1) proteins, Streptavidin-HRP, and assay buffers for 100 reactions.

The assay requires only a few steps. First, Fc (IgG1) is coated on a 96-well plate overnight. After blocking, the protein is pre-incubated with the inhibitor or neutralizing antibody. Upon subsequent incubation with Biotin-FcRn, 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.

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.

The function of FcRn can be exploited by engineering therapeutic antibodies to increase their binding to FcRn, thereby improving their half-life and therapeutic efficacy. For example, an antibody cocktail that contains Fc mutations and an extended half-life (Evusheld) is used to treat COVID-19. The first-in-class drug, Enbrel, a TNF-alpha/Fc fuses Fc portions to a therapeutic protein to increase their half-life. There are now several other drugs in clinical using similar strategies.

Conversely, 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)

This kit is useful for screening inhibitors of **FcRn** binding to **Fc (IgG1).**

Supplied Materials

Catalog #	Name	Amount	Storage
71456	IgG1, Fc (Human)*	2 x 5 µg	-80°C
71283	FcRn (FCGRT/B2M), His-Avi-Tag, Biotin labeled*	2 x 5 µg	-80°C
79311	3X Immuno Buffer 1	50 ml	-20°C
78502	Blocking Buffer 6	50 ml	+4°C
79742	Streptavidin-HRP	5 µl	+4°C
79651	HRP Colorimetric Substrate	10 ml	+4°C
79964	Clear 96-well microplate	1	Room Temp

**The initial concentration of both FcRn and Fc (IgG1) 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)
Rotating or rocker platform
UV/Vis spectrophotometer microplate reader capable of reading absorbance at 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

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

Assay Protocol

- All samples and controls should be tested in duplicate.
- 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.

Day 1 - Coating the plate with Fc (IgG1) protein:

1. Thaw **Fc (IgG1) protein** on ice. Briefly spin the tube to recover the full contents.

2. Calculate the amount of protein needed for the assay, aliquot the remaining protein into single use aliquots, and immediately store at -80°C.

⚠ *Note: **Fc (IgG1) protein** is very sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles.*

3. Dilute **Fc (IgG1) protein** to 2 µg/ml in PBS.

4. Add 50 µl of diluted **Fc (IgG1) protein** solution to each well.

5. Add 50 µl of PBS to "no coat" wells.

6. Incubate at 4°C overnight

Day 2 - Testing Compound:

1. Prepare **1x Immuno Buffer** by diluting **3x Immuno Buffer** in distilled water by adding one volume of stock Immuno Buffer to 2 volumes of water for a 3-fold dilution.

2. After the overnight coating, discard the solution by inverting the plate and tapping onto clean paper towels.

3. Wash the plate three times with 100 µl/well of **1x Immuno Buffer 1**. Discard the solution by inverting the plate and tapping onto clean paper towels.

4. Block by adding 100 µl of **Blocking Buffer 6** to each well. Incubate for 1 hour at room temperature with slow shaking.

5. Discard the solution by inverting the plate and tapping onto clean paper towels to dry.

****Note: there are two methods for steps 6-10 depending on your inhibitor****

<u>If testing an anti-Fc (IgG1) antibody as inhibitor or blocker, follow Steps 6-10 below:</u>

6. Prepare dilutions of **neutralizing anti-Fc (IgG1)** antibody or blocker in **Blocking Buffer 6** to the desired concentrations (it is recommended to use serial dilutions). Prepare enough for 50 µl per well.

7. Add 50 µl of the diluted antibody to the "Test Inhibitor" wells. To wells designated "Blank" and "Positive Control", add 50 µl of **Blocking Buffer 6.**

    a. Incubate the plate for 30 minutes (up to 1 hour) at room temperature with slow rotation.

8. Thaw the **FcRn-Biotin** on ice and dilute it to 1.5 µg/ml in **Blocking Buffer 6.**

a. Prepare only the amount required for the assay (50 µl/well); store the remaining **Biotin-FcRn** undiluted at -80°C.

⚠ *Note: **Biotin-FcRn** is very sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles.*

9. After the antibody incubation, add an equal volume (50 µl) of diluted **FcRn-Biotin** to the wells labeled "Test Inhibitor" and "Positive Control".

a. Add 50 µl **Blocking Buffer 6** to the wells labeled "Blank".

Note: At this step, there should be a total of 100 µl in each well.

b. Incubate the plate at room temperature for another 1 hour with slow rotation.

	Blank	Positive Control	Test Inhibitor
Blocking Buffer 6	100 µl	50 µl	-
Test antibody	-	-	50 µl
FcRn-Biotin (1.5 µg/ml)	-	50 µl	50 µl
Total	**100 µl**	**100 µl**	**100 µl**

10. After 1 hour, discard the solution and wash the plate three times with **1x Immuno Buffer 1**.

⚠ **Proceed to Detection: Step 11.**

<u>If testing a small molecule inhibitor, follow steps 6-10 below:</u>

6. Prepare the test inhibitor:

a. For a titration, prepare serial dilutions at concentrations 10-fold higher than the desired final concentrations. Prepare enough for 5 µl/well. The final volume of the reaction is 50 µl.

Without DMSO

b. If the Test Inhibitor is water-soluble, prepare serial dilutions in distilled water, 10-fold more concentrated than the desired final concentrations.

With DMSO

b. If the Test inhibitor is soluble in DMSO,

c. Prepare the test inhibitor at 100-fold the highest desired concentration in DMSO, then dilute the inhibitor 10-fold in distilled water to prepare the highest concentration of the 10-fold intermediate dilutions. The concentration of DMSO is now 10%. (*e.g.* To test a compound at 10 µM, prepare the inhibitor in DMSO at 1 mM. Then make a 10-fold dilution in distilled water to obtain a 100 µM solution in 10% DMSO)

d. Using 10% DMSO diluted in water, prepare serial dilutions of the Test Inhibitor at 10-fold the desired final concentrations to keep the concentration of DMSO constant.

e. For controls, prepare 10% DMSO in distilled water (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%

7. Add 5 µl to each well labeled "Test Inhibitor".

a. To the "Positive Control" and "Blank" wells, add 5 µl of the diluent solution without inhibitor (e.g. 10% DMSO solution in water) so that all wells contain the same amount of DMSO.

Caution! – It is highly recommended that the final DMSO concentration does not exceed 1%. Organic solvents other than DMSO have not been validated in this assay, so use of these solvents must be optimized by the user.

8. Thaw the **Biotin-FcRn** on ice, and dilute it in **Blocking Buffer 6** at 1.5 µg/ml.

a. Prepare only the amount required for the assay; store the remaining **Biotin-FcRn** undiluted, in aliquots, at -80°C.

*Note: **Biotin-FcRn** is very sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles.*

9. Add 20 µl of **Blocking Buffer 6** to the wells labeled "Blank".

a. Add 20 µl of diluted **Biotin-FcRn** to the wells labeled "Test Inhibitor" and "Positive Control".

b. Incubate the plate at room temperature for 1 hour with slow rotation.

	Blank	Positive Control	Test Inhibitor
Blocking Buffer 6	45 µl	25 µl	25 µl
Test Inhibitor	-	-	5 µl
Diluent solution (no inhibitor) or 10% DMSO	5 µl	5 µl	-
FcRn-Biotin (1.5 µg/ml)	-	20 µl	20 µl
Total	**50 µl**	**50 µl**	**50 µl**

10. After 1 hour, discard the solution and wash the plate three times with 100 µl of **1x Immuno Buffer 1**. Tap the plate onto clean paper towels to remove liquid.

Proceed to Detection: Step 11.

Detection:

11. Dilute **Streptavidin-HRP** 1000-fold with the **Blocking Buffer 6,** enough for 50 µl per well.

12. Add 50 µl of the **diluted Streptavidin-HRP** to each well and incubate the plate for 30 minutes at room temperature with slow rotation.

13. After 30 minutes, discard the solution and wash the plate three times.

14. Prepare enough “stop solution" 1N HCl (aqueous) for 100 µl per well.

    Note: alternatively, 2N H_2SO_4 or other compatible acidic solutions can be substituted.

15. Add 100 µl of the **Colorimetric HRP substrate** to each well and incubate the plate at room temperature until a blue color has developed in the ‘Positive Control’ wells.

    Note: This usually takes 1-5 minutes. The optimal incubation time may vary and should be determined empirically by the user. It is recommended that the reaction be stopped when the ‘Positive Control’ well is lower than ~ 1.0 absorbance at 450 nm (preferably ~ 0.6).

16. Once a blue color has developed in the ‘Positive Control’ wells, add 100 µl of **1N HCl stop solution** prepared above to every well. The blue color should turn yellow.

17. Read the absorbance at 450 nm using UV/Vis spectrophotometer microplate reader.

Example Results

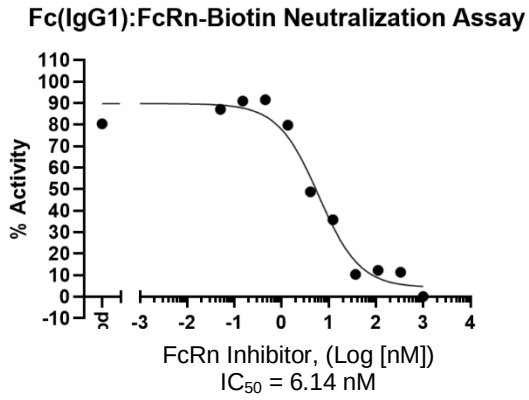

Figure 1. Inhibition of Fc (IgG1): FcRn binding by an FcRn (FCGRT/B2M) blocker.
The FcRn blocker (BPS Bioscience #101468) was evaluated using the Fc (IgG1): FcRn Inhibitor Screening Colorimetric Assay Kit. The blocker was serially diluted from 1 µM in 3-fold increments serial dilutions and tested using Fc (IgG1): FcRn using Inhibitor Screening Colorimetric Assay Kit (BPS Bioscience #78501).

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 absorbance in the assay. We recommend doing these in duplicate.

Troubleshooting Guide

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

References

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

Related Products

Products	*Catalog #*	*Size*
FcRn Blocker	101468	100 µg
FcRn (FCGRT/B2M), His-Avi-Tag, Biotin labeled HiP™	71283	25 µg/50 µg
FcRn (FCGRT/B2M), His-Avi-Tag	71285	100 µg/1 mg
Fc (IgG1) HiP™	71456	200 µg