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 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.

The assay mechanism is described next. 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)

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	10 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 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%.

Assay Protocol

- All samples and controls should be tested in duplicate.
- The assay should “Non-Coated Condition”, “Blank”, “Positive Control” and “Test Inhibitor” 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.

Step 1 - Plate coating with Fc (IgG1) protein

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

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

2. Dilute **Fc (IgG1)** protein to 2 µg/ml in PBS (50 µl/well).

   Note: Aliquot the remaining protein into single use aliquots, and immediately store at -80°C. Fc (IgG1) protein is very sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles.

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

4. Add 50 µl of PBS to "Non-Coated Condition" wells.

5. Incubate at 4°C overnight.

6. Prepare **1x Immuno Buffer** by diluting 3-fold **3x Immuno Buffer** with distilled water.

7. Tap the plate onto clean paper towel to remove the liquid.

8. Wash each well with 100 µl of 1x Immuno Buffer 1.

9. Tap the plate onto clean paper towel to remove the liquid.

10. Repeat steps 8 and 10 twice.

11. Adding 100 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 clean paper towel to remove the liquid.

14. Start your assay test immediately.

Step 2.1: Assay for detection of anti-Fc (IgG1) antibody as inhibitor or blocker activity

1. Prepare a serial dilution of anti-Fc (IgG1) antibody or blocker in Blocking Buffer 6 at the desired concentrations (50 µl/well).

2. Add 50 µl of the diluted antibody to the "Test Inhibitor" wells.

3. Add 100 µl of Blocking Buffer 6 to the "Blank" wells.

4. Add 50 µl of Blocking Buffer 6 to the "Positive Control" wells.

5. Incubate the plate for 30 minutes (up to 1 hour) at RT with gentle agitation.

6. Thaw the **FcRn-Biotin** on ice. Briefly spin the tube to recover the full content.

7. Dilute FcRn-Biotin to 1.5 µg/ml in Blocking Buffer 6 (50 µl/well).

   Note: Prepare only the amount required for the assay. Store the remaining FcRn-Biotin undiluted at -80°C Biotin-FcRn is very sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles.

8. Add 50 µl of diluted FcRn-Biotin to the "Test Inhibitor" and "Positive Control" wells.

9. Incubate the plate at RT for 1 hour with gentle agitation.

10. Wash the plate three times with 1x Immuno Buffer 1.

11. Dilute **Streptavidin-HRP** 1000-fold with the Blocking Buffer 6 (50 µl/well).

12. Add 50 µl of the diluted Streptavidin-HRP to each well.

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

14. Wash the plate three times with 100 µl of 1x Immuno Buffer 1.

15. Prepare 1N HCl (aqueous) (100 µl/well). This is the **Stop Solution**.

16. Add 100 µl of **Colorimetric HRP Substrate** to each well.

17. Incubate the plate at RT until the 'Positive Control' wells' solution becomes blue.

    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).

18. Add 100 µl of Stop Solution to every well. The blue colored solution will turn yellow.

19. Read the absorbance at λ=450 nm using an UV/Vis spectrophotometer microplate reader.

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

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Step 2.2: Assay for detection of small molecule inhibitor or blocker activity

1. Prepare the test inhibitor (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.

   1.1 If the Test Inhibitor is water-soluble, prepare serial dilutions in distilled water at concentrations 10-fold higher than the desired final concentrations. Distilled water is the Diluent Solution.

   OR

   1.2. If the Test inhibitor 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 distilled water to prepare the highest concentration of the serial dilutions. The concentration of DMSO is now 10%.

   Using distilled water 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 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%.

2. Add 5 µl of diluted Test Inhibitor to each well labeled “Test Inhibitor”.

3. Add 5 µl of the Diluent Solution to the “Positive Control” and “Blank” wells.

4. Thaw **FcRn**-**Biotin** on ice. Briefly spin the tube to recover the full content.

5. Dilute FcRn-Biotin to 1.5 µg/ml in Blocking Buffer 6 (20 µl/well).

   Note: Prepare only the amount required for the assay. Store the remaining FcRn-Biotin undiluted at -80°C Biotin-FcRn is very sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles.

6. Add 20 µl of diluted FcRn-Biotin to the wells labeled “Test Inhibitor” and “Positive Control”.

7. Add 25 µl of Blocking Buffer 6 to the “Test Inhibitor” and “Positive Control” wells.

8. Add 45 µl of Blocking Buffer 6 to the “Blank” wells.

9. Incubate the plate at RT for 1 hour with gentle agitation.

10. Wash the plate three times with 100 µl of 1x Immuno Buffer 1.

11. Dilute **Streptavidin-HRP** 1000-fold with the Blocking Buffer 6 (50 µl/well).

12. Add 50 µl of the diluted Streptavidin-HRP to each well.

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

14. Wash the plate three times with 1x Immuno Buffer 1.

15. Prepare 1N HCl (aqueous) (100 µl/well). This is the **Stop Solution**.

16. Add 100 µl of **Colorimetric HRP Substrate** to each well.

17. Incubate the plate at RT until the 'Positive Control' wells' solution becomes blue.

    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).

18. Add 100 µl of Stop Solution to every well. The blue colored solution will turn yellow.

19. Read the absorbance at λ=450 nm using an UV/Vis spectrophotometer microplate reader.

	Blank	**Positive Control**	**Test Inhibitor**
Blocking Buffer 6	45 µl	25 µl	25 µl
Test Inhibitor	-	-	5 µl
Diluent Solution	5 µl	5 µl	-
Diluted FcRn-Biotin (1.5 µg/ml)	-	20 µl	20 µl
Total	**50 µl**	**50 µl**	**50 µl**

Example Results

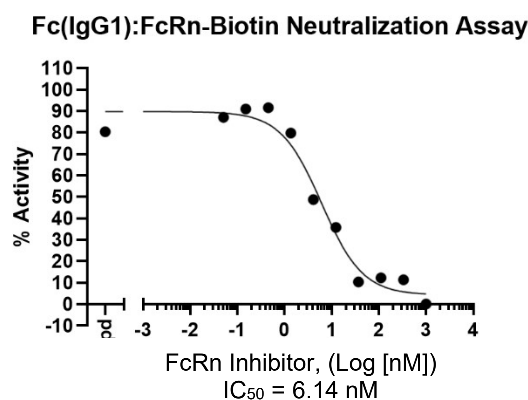

Figure 1. Inhibition of Fc (IgG1): FcRn binding by FcRn (FCGRT/B2M) blocker.
Fc (IgG1): FcRn binding was evaluated in the presence of increasing concentrations of FcRn (FCGRT/B2M) Blocker (BPS Bioscience #101468). Results are expressed as percent activity, in which the binding activity in the absence 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 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

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