Description

The FcRn: IgG or HSA Inhibitor Screening Chemiluminescent Assay Kit is designed for screening and profiling biologics targeting the interaction between human FcRn (Neonatal Fc receptor for IgG) and Human Serum Albumin (HSA) or human FcRn and human Fc (IgG1), respectively. This kit comes in a convenient 96-well format, with enough Fc (IgG1) (amino acids 100-330), Biotinylated HSA and His-tagged FcRn (FCGRT/B2M) (amino acids 24-297 of FCGRT and 21-119 of B2M), as well as anti-His detection antibody, Streptavidin-HRP, and assay buffers for 100 reactions.

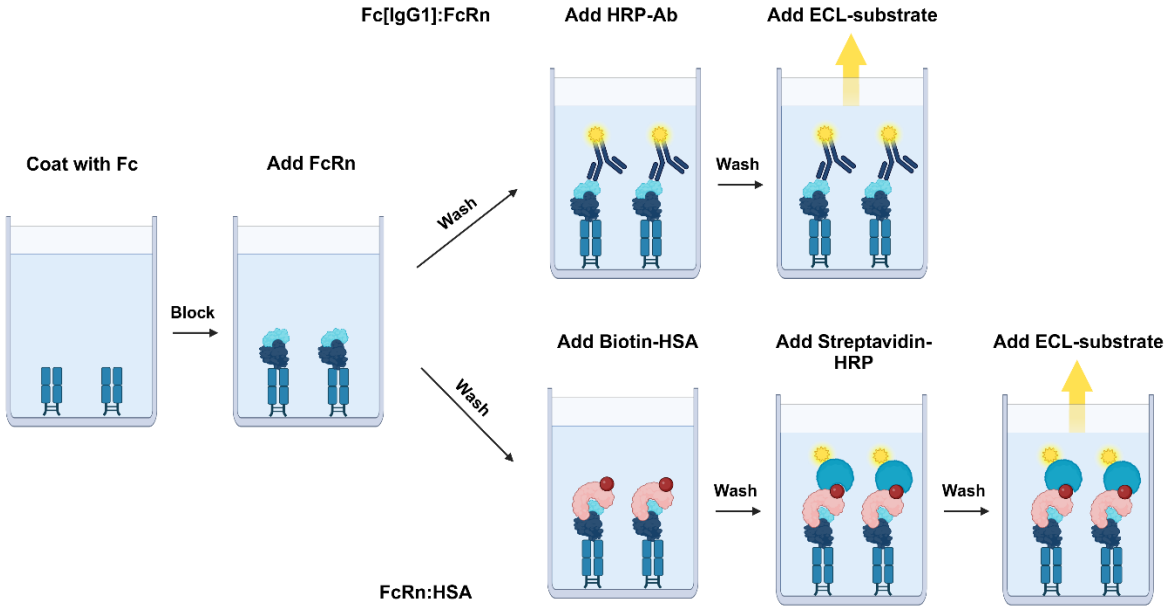

Figure.1 Assay principle

This universal assay kit provides flexibility as it allows to choose between two protocols for profiling biologics targeting the Fc(IgG1):FcRn or FcRn:HSA interactions, respectively.

Protocol A: Fc (IgG1) is coated on a 96-well plate overnight. The plate is incubated with an inhibitor or neutralizing antibody together with FcRn, washed, and treated with anti-His detection antibody to assess the fraction of His-tagged FcRn bound to the Fc(IgG1). Addition of an HRP-conjugated substrate produces chemiluminescence, which is proportional to the amount of FcRn bound to Fc(IgG1). **Protocol B:** Fc (IgG1) is coated on a 96-well plate overnight. FcRn is added and captured by the coated Fc (IgG1). Next, the plate is incubated with an inhibitor or neutralizing antibody together with biotinylated HSA. Finally, the plate is washed and treated with Streptavidin-HRP, followed by addition of an HRP substrate to produce chemiluminescence. The chemiluminescence signal is proportional to the binding of HSA to FcRn.

Background:

Neonatal Fc receptor for IgG (FcRn) is a heterodimeric protein consisting 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-

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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)

Screen or titrate neutralizing antibodies or blockers of FcRn binding to HSA, or Fc(IgG1) binding to FcRn, in high throughput screening (HTS) applications.

Supplied Materials

Catalog #	Name	Amount	Storage
71456	IgG1, Fc (Human)*	10 µg	-80°C
	Human Serum Albumin (HSA), Biotin-Labeled*	20 µg	-80°C
71285	FcRn (FCGRT/B2M), His-Tag*	10 µg	-80°C
78502	Blocking Buffer 6	50 ml	+4°C
82646	3x Acidic FcRn Wash Buffer	50 ml	-20°C
82609	5X FcRn Binding Buffer 2	1.5 ml	-20°C
78033	Anti-His-HRP-Conjugated Detection Antibody	10 µl	-80°C
79742	Streptavidin-HRP	10 µl	+4°C
79670	ELISA ECL Substrates A	6 ml	Room Temp
	ELISA ECL Substrates B	6 ml	Room Temp
79837	White 96-well strip microplate	1	Room Temp

**The initial concentration of 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.

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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.
- This assay should have "Blank" (non-coated control), "Positive Control" and "Test Compound" conditions.
- Follow protocol A if studying IgG1 binding to FcRn.
- Follow protocol B if studying FcRn binding to HSA.
- Step 1 (plate coating) is the same for protocol A and B.
- We recommend using ADM31 (Antibodies Online #ABIN1774762) as an internal control for inhibition of HSA binding to FcRn, and FcRn (FCGRT/B2M) Blocker (#101468) for inhibition of IgG binding to FcRn. 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 proteins 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: Coating the plate with Fc (IgG1) protein.

Coat the plate one day prior to running your samples.

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 in PBS (50 µl/well).
3. Add 50 µl of **diluted Fc (IgG1)** protein solution to each well, except the "Blank (Non-Coated Control)" wells.
4. Add 50 µl of PBS to the "Blank" wells.
5. Incubate at 4°C overnight.
6. Prepare **1x Acidic FcRn Wash Buffer** by diluting **3x Acidic FcRn Wash Buffer** 3-fold 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 150 µl of **Blocking Buffer 6** to every well.

11. Incubate for 1 hour 30 minutes 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.

Protocol A: Fc(IgG1) binding to FcRn

Step 2: Reaction

1. Prepare **1x FcRn Binding Buffer 2** by diluting 5-fold the **5x FcRn Binding Buffer 2** with distilled water.
2. Prepare a serial dilution of the Neutralizing Antibody or Blocker being tested in 1x FcRn Binding Buffer 2 at a concentration 2x higher than the desired testing concentrations (25 µl/well).
3. Add 25 µl of the diluted antibody/blocker to the "Test Compound" wells.
4. Add 25 µl of 1x FcRn Binding Buffer 2 to the "Blank" and "Positive Control" wells**.**
5. Thaw **FcRn** on ice.
6. Dilute **FcRn** to 4 ng/µl with **1x FcRn Binding Buffer 2** (25 µl/well).
7. Add 25 µl of **diluted FcRn** to all the wells.
8. Incubate the plate at RT for 1 hour with gentle agitation.
9. Wash the plate three times with **1x Acidic FcRn Wash Buffer**.

	Blank (Non-Coated Control**)**	**Positive Control**	**Test Compound**
1x FcRn Binding Buffer 2	25 µl	25 µl	-
Test Inhibitor/ Blocker	-	-	25 µl
Diluted FcRn (4 ng/µl)	25 µl	25 µl	25 µl
Total	**50 µl**	**50 µl**	**50 µl**

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Step 3: Detection

1. Dilute **Anti-His HRP-Conjugated Detection Antibody** 2000-fold with **Blocking Buffer 6** (50 µl/well).
2. Add 50 µl of the diluted **Anti-His HRP-Conjugated Detection Antibody** to each well.
3. Incubate the plate for 1 hour at RT with gentle agitation.
4. After 1 hour, discard the solution and wash the plate three times with **1x Acidic FcRn Wash Buffer**.
5. Just before use, prepare a mix (100 µl/well): N wells x (50 µl ELISA ECL Substrate A + 50 µl ELISA ECL Substrate B).
6. Add 100 µl of mix to each well.

   Note: Discard any unused chemiluminescent mix after use.
7. Immediately read the plate in a luminometer or plate reader capable of reading chemiluminescence.
8. The "Blank" value should be subtracted from all readings.

Protocol B: HSA binding to FcRn

Step 2: Reaction

1. Prepare **1x FcRn Binding Buffer 2** by diluting 5-fold the **5x FcRn Binding Buffer 2** with distilled water.
2. Dilute **FcRn** to 2 ng/µl with **1x FcRn Binding Buffer 2** (50 µl/well).
3. Add 50 µl of **diluted FcRn** to all wells.
4. Incubate the plate at RT for 1 hour with gentle agitation.
5. Wash the plate three times with **1x Acidic FcRn Wash Buffer**.
6. Prepare a serial dilution of the Neutralizing Antibody or Blocker being tested in 1x FcRn Binding Buffer 2 at the desired testing concentrations (25 µl/well).
7. Add 25 µl of the diluted antibody to the "Test Compound" wells.
8. Add 25 µl of **1x FcRn Binding Buffer 2** to the "Blank" and "Positive Control" wells**.**

   Note: If needed pre-incubate the plate for 30 minutes (up to 1 hour) at RT with gentle agitation.
9. Thaw **Biotin-HSA** on ice.

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10. Dilute **Biotin-HSA** to 8 ng/µl with **1x FcRn Binding Buffer 2** (25 µl/well).

11. Add 25 µl of **diluted Biotin-HSA** to all the wells.

	Blank (Non-Coated Control**)**	**Positive Control**	**Test Compound**
Diluted FcRn (2 ng/µl)	50 µl	50 µl	50 µl
Incubate at RT for 1 hour and wash			
1x FcRn Binding Buffer 2	25 µl	25 µl	-
Test inhibitor/biologic	-	-	25 µl
Diluted Biotin-HSA (8 ng/µl)	25 µl	25 µl	25 µl
Total	**50 µl**	**50 µl**	**50 µl**

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

13. Wash the plate three times with **1x Acidic FcRn Wash Buffer**.

Step 3b: Detection

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

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

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

4. After 1 hour, discard the solution and wash the plate three times with **1x Acidic FcRn Wash Buffer**.

5. Just before use, prepare a mix (100 µl/well): N wells x (50 µl ELISA ECL Substrate A and 50 µl ELISA ECL Substrate B).

6. Add 100 µl of mix to each well.

   Note: Discard any unused chemiluminescent mix after use.

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

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

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

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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

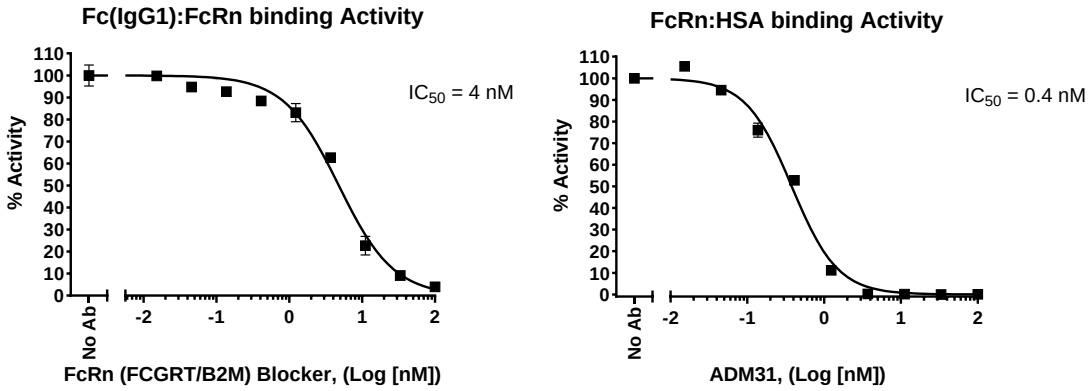

Figure 2. Inhibition of FcRn binding to Fc(IgG1) or HSA by neutralizing molecules.
Left Panel: The inhibition of FcRn binding to Fc(IgG1) was measured in the presence of increasing concentrations of FcRn (FCGRT/B2M) Blocker (#101468), as described in Protocol A. Right Panel: Inhibition of the binding of FcRn to HSA was evaluated in the presence of increasing concentrations of the antibody ADM31 (Antibodies Online #ABIN1774762), as described in Protocol B. Results are expressed as the percent of "Positive control" (activity in the absence of inhibitor, set at 100%). The "Blank" value was subtracted from all other values.

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

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Dall'Acqua W.F., *et al.*, 2002 *J Immunol.* 169(9): 5171-80.
Sand K.M.K., *et al.*, 2014 *J Biol Chem.* 289 (24):17228-39.

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Related Products

Products	*Catalog #*	*Size*
FcRn: IgG Recycling HMEC-1 Cell Pool	82163	2 vials
Fc(IgG1):FcRn Inhibitor Screening TR-FRET Assay Kit	82653	384 reactions
Fc(IgG1):FcRn Inhibitor Screening Colorimetric Assay Kit	78501	96 reactions/ 384 reactions
Human IgG Chemiluminescence ELISA Kit	82611	96 reactions
Human IgG Colorimetric ELISA Kit	82612	96 reactions

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