Description

The FcRn:HSA Binding Chemiluminescent Assay Kit is designed for screening and profiling neutralizing antibodies or blockers of the interaction between Human Serum Albumin (HSA) and human FcRn. This kit comes in a convenient 96-well format, with Biotinylated HSA and FcRn (FCGRT/B2M) (amino acids 24-297 of FCGRT and 21-119 of B2M), Streptavidin-HRP, and assay buffers for 100 reactions.

The assay requires only a few steps. First, FcRn is coated on a 96-well plate overnight. After blocking, the protein is pre-incubated with the neutralizing antibody or blocker. Upon subsequent incubation with Biotin-HSA, the plate is treated with Streptavidin-HRP followed by addition of an HRP substrate to produce chemiluminescence, which can then be measured using a chemiluminescence 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)

Screen or titrate neutralizing antibodies or blockers of FcRn binding to Human Serum Albumin (HSA) in high throughput screening (HTS) applications.

Supplied Materials

Catalog #	Name	Amount	Storage
	Human Serum Albumin (HSA), Biotin-Labeled *	50 µg	-80°C
71285	FcRn (FCGRT/B2M), His-Tag*	250 µg	-80°C
78502	Blocking Buffer 6	25 ml	+4°C
	5X FcRn Binding Buffer 2	1.5 ml	-20°C
79742	Streptavidin-HRP	10 µl	+4°C
79670	ELISA ECL Substrates A and B (2 components)	6 ml each	Room Temp
79837	White 96-well strip microplate	1	Room Temp

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

Materials Required but Not Supplied

- PBS buffer (Phosphate Buffer Saline)
- PBST Buffer (1X PBS containing 0.05% Tween-20), pH 5.5.
- 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

- This kit is not suitable for screening small molecules inhibitors or peptides.
- 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.
- This assay should have “Blank” (uncoated wells, for background determination), “Positive Control” and “Test Compound” conditions.

- If the assay is going to be used more than once, aliquot remaining undiluted reagents into single-use aliquots (volumes lower than 5 µl are not recommended) depending on how many times the assay plate will be used. Store the aliquots at -80°C or as recommended for each reagent.

Day 1
Step 1: Coating the plate with FcRn protein.

1. Thaw **FcRn** protein on ice. Briefly spin the tube to recover the full content.
2. Dilute **FcRn** to 50 µg/ml in PBS (50 µl/well).
3. Add 50 µl of diluted FcRn protein solution to each well, except "Blank" wells.
4. Add 50 µl of PBS to the "Blank" wells.
5. Incubate at 4°C overnight.

Day 2
Step 1: Compound Testing.

1. Discard the solution by inverting the plate and tapping onto clean paper towels.
2. Wash the plate three times with 200 µl/well of PBST Buffer, pH 5.5.
3. Discard the solution by inverting the plate and tapping onto clean paper towels.
4. Add 150 µl of **Blocking Buffer 6** to each well.
5. Incubate for 1 hour 30 minutes at Room Temperature (RT) with gentle agitation.
6. Discard the solution by inverting the plate and tapping onto clean paper towels to dry.
7. Prepare **1x FcRn Binding Buffer 2** by diluting 5-fold the **5x FcRn Binding Buffer 2** with distilled water.
8. Prepare a serial dilution of Neutralizing Antibody or Blocker being tested in 1x FcRn Binding Buffer 2 at the desired testing concentrations (25 µl/well).
9. Add 25 µl of the diluted antibody to the "Test Inhibitor" wells.
10. Add 25 µl of 1x FcRn Binding Buffer 2 to the "Blank" and "Positive Control" wells**.**
11. Incubate the plate for 30 minutes (up to 1 hour) at RT with gentle agitation.
12. Thaw **Biotin-HSA** on ice.
13. Dilute biotin-HSA to 10 µg/ml with 1x FcRn Binding Buffer 2 (25 µl/well).

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

14. Add 25 µl of diluted ***Biotin-HSA*** to all the wells.
15. Incubate the plate at RT for 1 hour 30 minutes with gentle agitation.
16. Wash the plate three times with PBST Buffer, pH 5.5.

	Blank (Uncoated)	Positive Control	Test Compound
1x FcRn Binding Buffer 2	25 µl	25 µl	-
Test Compound	-	-	25 µl
Diluted Biotin-HSA (10 µg/ml)	25 µl	25 µl	25 µl
Total	**50 µl**	**50 µl**	**50 µl**

Step 2: 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.
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 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

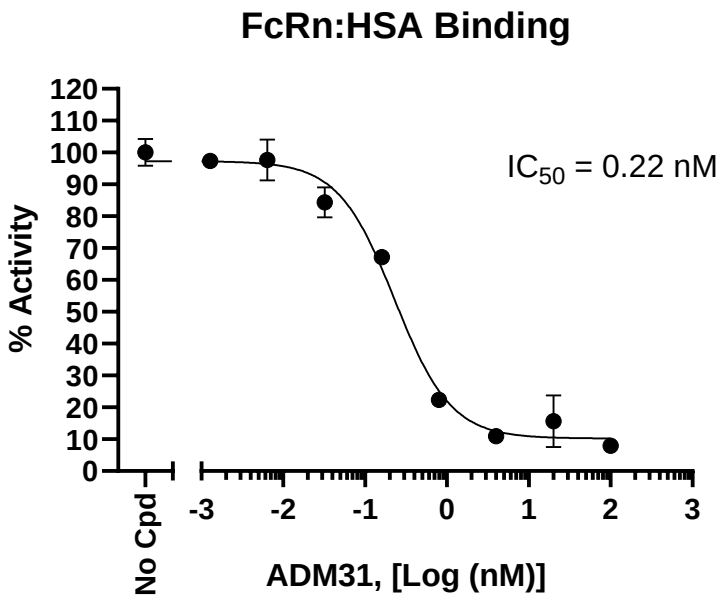

Figure 1. Inhibition of FcRn:HSA binding by the antibody ADM31.
The inhibition of FcRn binding to HSA was evaluated in the presence of increasing concentrations of antibody ADM31. The antibody was serially diluted, starting at 100 nM, in 5-fold increments.

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
Chaudhury C., *et al*., 2006 *Biochemistry.* 45 (15): 4983-90.
Dall'Acqua W.F., *et al*., 2002 *J Immunol.* 169(9): 5171-80.

Related Products

Products	*Catalog #*	*Size*
FcRn (FCGRT/B2M), His-Avi-Tag, Biotin-Labeled, HiP™ Recombinant	71283	25 µg/50 µg
Fc(IgG1):FcRn Inhibitor Screening Colorimetric Assay Kit	78501	96 reactions
FcRn (FCGRT/B2M) Blocker	101468	50 µg/100 µg

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