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Produktinformation

- Forschungsprodukte & Biochemikalien
- Zellkultur & Verbrauchsmaterial
- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Description

The HSA:FcRn Neutralizing Antibody Screening Chemiluminescent Assay Kit is designed for the screening and profiling of neutralizing antibodies or blockers of the interaction between Human Serum Albumin (HSA) and human FcRn (Neonatal Fc receptor for IgG). This kit comes in a convenient 384-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 400 reactions.

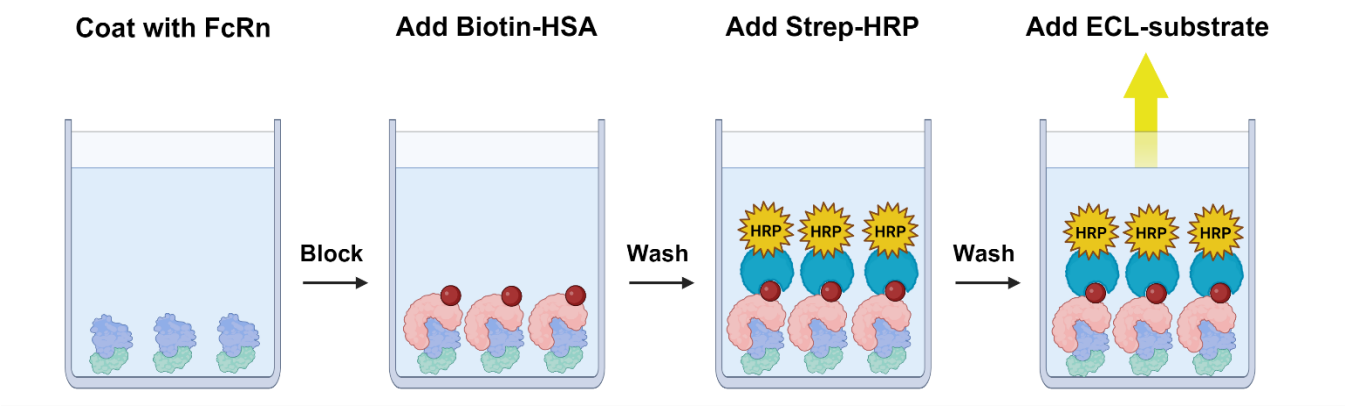

Figure 1: Illustration of the mechanism behind the HSA:FcRn Neutralizing Antibody Screening Chemiluminescent Assay Kit.

A 384-well plate is coated with FcRn protein. After blocking, the plate is pre-incubated with a blocker or neutralizing antibody. Upon subsequent incubation with Biotin-HSA, the plate is treated with Streptavidin-HRP followed by addition of the ELISA ECL substrate to produce chemiluminescence that can be measured using a chemiluminescence reader. The chemiluminescence signal is proportional to the binding of HSA to FcRn.

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 *	2 x 50 µg	-80°C
71285	FcRn (FCGRT/B2M), His-Tag*	2 x 250 µg	-80°C
82646	3x Acidic FcRn Wash Buffer	2 x 50 ml	-20°C
82609	5x FcRn Binding Buffer 2	2 x 1.5 ml	-20°C
78502	Blocking Buffer 6	2 x 50 ml	+4°C
79742	Streptavidin-HRP	2 x 10 µl	+4°C
79670	ELISA ECL Substrates A and B (2 components)	2 x 6 ml each	Room Temp
78188	384-well white 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 buffer (Phosphate Buffer 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.

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.
- 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.

- We recommend using ADM31 (Genovac #GM-0808) 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).

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 ng/µl in PBS (25 µl/well).
3. Add 25 µl of diluted FcRn protein solution to each well, except the "Non-Coated Control" wells.
4. Add 25 µl of PBS to the "Non-Coated Control" wells.
5. Incubate at 4°C overnight.

Day 2

Step 1: Compound Testing.

1. Prepare **1x Acidic FcRn Wash Buffer** by diluting 3-fold **3x Acidic FcRn Wash Buffer** with distilled water.
2. Tap the plate onto a clean paper towel to remove the liquid.
3. Wash the plate three times with 50 µl/well of 1x Acidic FcRn Wash Buffer.
4. Tap the plate onto a clean paper towel to remove the liquid.
5. Add 50 µl of **Blocking Buffer 6** to each well.
6. Incubate for 1 hour 30 minutes at Room Temperature (RT) with gentle agitation.
7. Discard the solution by inverting the plate and tapping onto clean paper towels to dry.
8. Wash the plate three times with 50 µl/well of 1x Acidic FcRn Wash Buffer.
9. Tap the plate onto a clean paper towel to remove the liquid.
10. Prepare **1x FcRn Binding Buffer 2** by diluting 5-fold the **5x FcRn Binding Buffer 2** with distilled water.
11. Prepare a serial dilution of Neutralizing Antibody or Blocker being tested in 1x FcRn Binding Buffer 2 at concentrations 2-fold higher than the desired final concentrations (12.5 µl/well).

12. Add 12.5 µl of the diluted antibody to the "Test Compound" wells.
13. Add 12.5 µl of 1x FcRn Binding Buffer 2 to the "Non-Coated Control" and "Positive Control" wells**.**
14. Add 25 µl of 1x FcRn Binding Buffer 2 to the "Blank" wells**.**
15. Incubate the plate for 30 minutes at RT with gentle agitation.
16. Thaw **Biotin-HSA** on ice. Briefly spin the tube to recover the full content.
17. Dilute Biotin-HSA to 10 ng/µl with 1x FcRn Binding Buffer 2 (12.5 µl/well).
18. Add 12.5 µl of diluted Biotin-HSA to the "Non-Coated Control", "Positive Control" and "Test Compound" wells.
19. Incubate the plate at RT for 1 hour 30 minutes with gentle agitation.
20. Wash the plate three times with 50 µl/well of 1x Acidic FcRn Wash Buffer.

	Blank	**Non-Coated Control**	**Positive Control**	**Test Compound**
1x FcRn Binding Buffer 2	25 µl	12.5 µl	12.5 µl	-
Test Compound	-	-	-	12.5 µl
Pre-incubate 30 minutes at RT				
Diluted Biotin-HSA (10 ng/µl)	-	12.5 µl	12.5 µl	12.5 µl
Total	**25 µl**	**25 µl**	**25 µl**	**25 µl**

Step 2: Detection

1. Dilute **Streptavidin-HRP** 1000-fold with Blocking Buffer 6 (25 µl/well).
2. Add 25 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 50 µl/well of 1x Acidic FcRn Wash Buffer.
5. Just before use, prepare a mix (50 µl/well): N wells x (25 µl ELISA ECL Substrate A and 25 µl ELISA ECL Substrate B).
6. Add 50 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

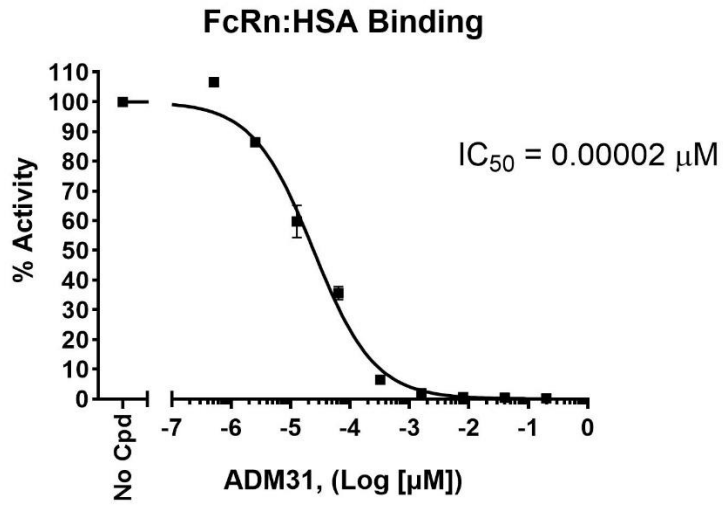

Figure 2. Inhibition of FcRn:HSA binding by the antibody ADM31.
The inhibition of FcRn binding to HSA was evaluated in the presence of increasing concentrations of antibody ADM31 (Genovac #GM-0808). The antibody was serially diluted, starting at 200 nM, in 5-fold increments.

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.
Sand K.M.K., *et al.*, 2014 *J Biol Chem*. 289 (24):17228-39.

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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