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Produktinformation

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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Description

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

The assay requires only a few steps. First, HSA is coated on a 96-well plate overnight. After blocking, the protein is pre-incubated with the inhibitor. Upon subsequent incubation with Biotin-FcRn, 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 small molecule inhibitors or peptides blocking FcRn binding to Human Serum Albumin (HSA) in high throughput screening (HTS) applications.

Supplied Materials

Catalog #	Name	Amount	Storage
	Human Serum Albumin (HSA)*	50 µg	-80°C
71283	FcRn Complex (FCGRT/B2M), His-Avi-Tag, Biotin-Labeled, HiP™*	25 µg	-80°C
78502	Blocking Buffer 6	25 ml	+4°C
	5X FcRn Binding Buffer 1	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 neutralizing antibodies.
- DMSO concentration in the final reaction should be ≤1%.

Assay Protocol

- All samples and controls should be tested in duplicate.
- For small molecule inhibitors, pre-incubation may also be beneficial, depending on the experimental conditions.
- 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 HSA protein.

1. Thaw **HSA protein** on ice. Briefly spin the tube to recover the full content.

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

2. Dilute **HSA protein** to 10 µg/ml in PBS (50 µl/well).
3. Add 50 µl of diluted **HSA protein** solution to each well, except "Blank" wells.
4. Add 50 µl of PBS to he "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 1** by diluting 5-fold the **5x FcRn Binding Buffer 1** with distilled water.

8. Prepare the Test Compound (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.

   8.1 If the Test Compound is water-soluble, prepare 10-fold more concentrated serial dilutions of the inhibitor than the desired final concentrations using the 1x FcRn Binding Buffer 1.

   For the positive and negative controls, use 1x FcRn Binding Buffer 1 (Diluent Solution).

   OR

   8.2 If the Test Compound is soluble in DMSO, prepare the test compound at a concentration 100-fold higher than the highest desired concentration in DMSO, then dilute the inhibitor 10-fold in 1x FcRn Binding Buffer 1 to prepare the highest concentration of the 10-fold intermediate dilutions. The concentration of DMSO is now 10%.

   Using 10% DMSO diluted in **1x FcRn Binding Buffer 1**, prepare serial dilutions of the Test Compound at 10-fold the desired final concentrations to keep the concentration of DMSO constant.

   For positive and negative controls, prepare 10% DMSO in 1x FcRn Binding Buffer 1 (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

   Note: The final concentration of DMSO should not exceed 1%.

9. Add 5 µl of the test compound dilutions to each well labeled “Test Compound”.

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

11. Add 20 µl of **1x FcRn Binding Buffer 1** to all the wells.

12. Incubate the plate for 30 minutes at RT with slow rotation.

13. Thaw the **Biotin-FcRn** on ice.

14. Dilute Biotin-FcRn to 10 µg/ml (25 µl/well) in **1x FcRn Binding Buffer 1.**

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

15. Add 25 µl of diluted **Biotin-FcRn** to all the wells.

16. Incubate the plate at RT for 1 hour 30 minutes with gentle agitation.

17. After the incubation, discard the solution and wash the plate three times with 200 µl of PBST Buffer, pH 5.5.

18. Tap the plate onto clean paper towels to remove liquid.

	Blank (Uncoated)	Positive Control	Test Inhibitor
1x FcRn Binding Buffer 1	20 µl	20 µl	20 µl
Test Inhibitor	-	-	5 µl
Diluent Solution	5 µl	5 µl	-
Diluted Biotin-FcRn (10 µg/ml)	25	25 µl	25 µl
Total	**50 µl**	**50 µl**	**50 µl**

Step 2:
Detection

1. Dilute **Streptavidin-HRP** 1000-fold with the **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 reagent after use.
7. Immediately read the plate in a luminometer or microtiter-plate 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

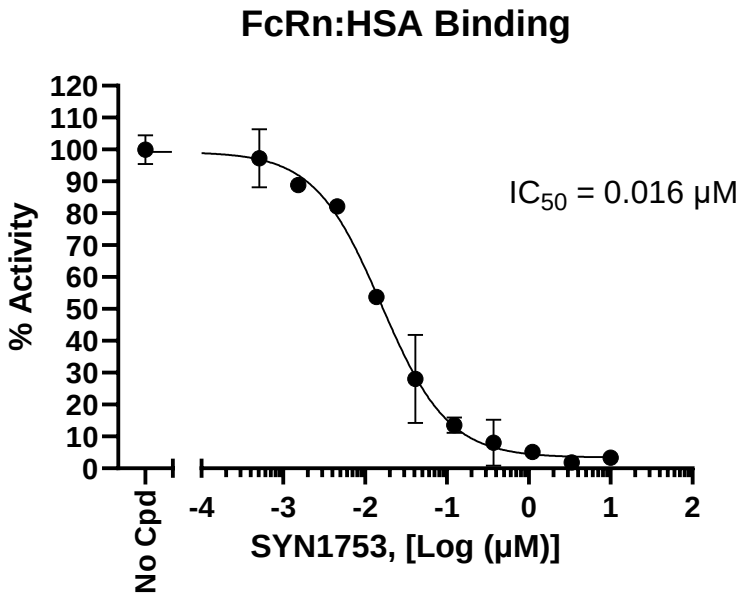

Figure 1. Inhibition of FcRn:HSA binding by SYN1753.
The inhibition of binding of FcRn to HSA was evaluated in the presence of increasing concentrations of the peptide SYN1753. The peptide was serially diluted, starting at 10 µM, in 3-fold increments serial dilutions.

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- Tag, Recombinant	71285	100 µg/1 mg
Fc (IgG1):FcRn Inhibitor Screening Colorimetric Assay Kit	78501	96 reactions
FcRn (FCGRT/B2M) Blocker	101468	50 µg/100 µg