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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Description

The Fc (IgG1): FcRn Inhibitor Screening TR-FRET Assay Kit is designed for screening and profiling of neutralizing antibodies or inhibitors of the interaction between Fc (IgG1) and human FcRn (Neonatal Fc receptor for IgG) using TR-FRET (Time-Resolved Fluorescence Resonance Energy Transfer). This kit requires no time-consuming washing steps and comes in a convenient 384-well format, with purified Biotinylated-FcRn complex (Fc receptor amino acids 24-297 and B2M amino acids 21-119) and europium-labeled (Eu) Fc (IgG1) protein (amino acids 100-330), Dye-labeled Acceptor, and assay buffer for 400 reactions.

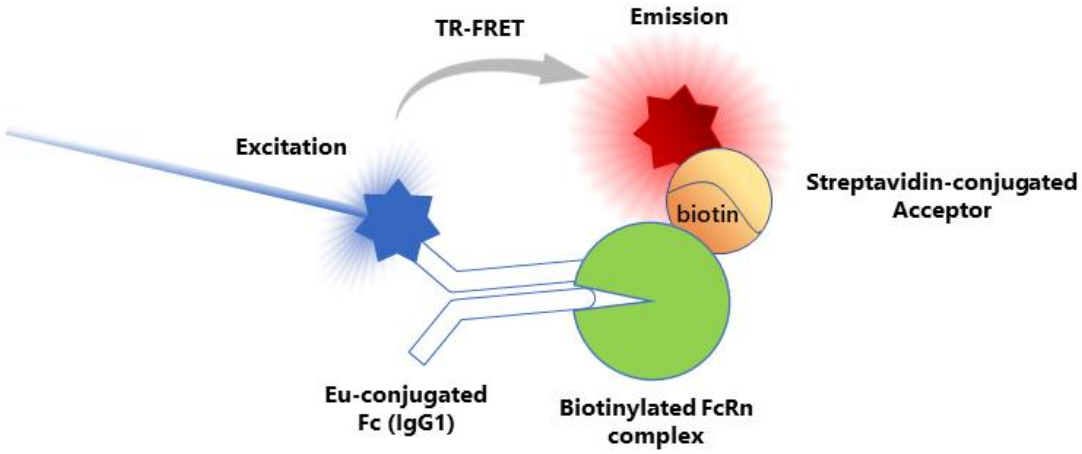

Figure 1. Illustration of the mechanism of the Fc (IgG1): FcRn Inhibitor Screening TR-FRET Assay Kit.

The assay procedure is straightforward and simple; a sample containing europium-labeled (Eu) Fc (IgG1), dye-labeled acceptor, biotin-labeled FcRn, and an inhibitor is incubated for one hour. Then, the fluorescence signal is measured at 620 nm and 665 nm using the TR-FRET module of a fluorescent microplate reader. When FcRn binds to Fc (IgG1), energy transfer occurs between the europium donor and the dye-labeled acceptor, producing a fluorescent signal. Inhibitors that block this interaction prevent energy transfer, reducing the fluorescent signal.

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. 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 inhibitors of FcRn binding to Fc (IgG1).

Supplied Materials

Catalog #	Name	Amount	Storage
101386	IgG1, Fc, Europium-Labeled*	5 µg	-80°C
71283	FcRn Complex (FCGRT/B2M), His-Avi-Tag, Biotin-Labeled*	5 µg	-80°C
82609	5x FcRn Binding Buffer 2	2 x 1.5 ml	-20°C
	Dye-Labeled Acceptor	2 x 10 µl	-20°C
79969	White 384-well 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

- Fluorescent microplate reader capable of measuring Time Resolved Fluorescence Resonance Energy Transfer (TR-FRET)
- Adjustable micropipettor and sterile filter tips
- Rotating or rocker platform

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 compatible with up to 1% final DMSO concentration.
- Compounds that are fluorescent may interfere with the results, depending on their spectral excitation and emission properties.
- It is recommended that the compound alone is tested to determine any potential interference of the compound on the assay results.

Assay Protocol

- All samples and controls should be tested in duplicate.
- The assay should include “Blank”, “Positive Control” and “Test Compound” wells.
- We recommend using FcRn (FCGRT/B2M) Blocker (#101468) 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).

Step 1

1. Prepare **1x FcRn Binding Buffer 2** by diluting 5-fold the **5x FcRn Binding Buffer 2** with distilled water.

2. Dilute **Dye-Labeled acceptor** 100-fold in 1x FcRn Binding Buffer 2 (5 µl/well).

   Note: Make only enough needed for the assay; store remaining stock solution in single use aliquots (minimum volume of 5 µl/ aliquot) at -20°C.

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

4. Dilute **Fc (IgG1)-Eu** protein to 1.6 ng/µl with 1x FcRn Binding Buffer 2 (5 µl/well).

5. Prepare a Master Mix (13 µl/well): N wells × (5 µl of diluted Dye-Labeled Acceptor + 5 µl of Diluted Fc (IgG1)-Eu + 3 µl of 1x FcRn Binding Buffer 2).

6. Add 13 µl of Master Mix to every well.

7. Prepare the Test Compound (2 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 20 µl.

   7.1 If the Test Compound is water-soluble, prepare serial dilutions in 1x FcRn Binding Buffer 2 at concentrations 10-fold higher than the desired final concentrations.

   OR

   7.2 If the Test Compound 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 1x FcRn Binding Buffer 2 to prepare the highest concentration of the serial dilutions. The concentration of DMSO is now 10%.

   Using 1x FcRn Binding Buffer 2 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 1x FcRn Binding Buffer 2 (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%.

8. Add 2 µl of Test Compound to each well designated “Test Compound”.

9. Add 2 µl of Diluent Solution to the “Positive Control” and “Blank” wells.

10. Incubate at Room Temperature (RT) for 15 minutes with gentle agitation.

Step 2

1. Thaw **FcRn Complex-biotin** on ice. Briefly spin the tube to recover its full content.
2. Dilute **FcRn Complex-biotin** to 2.5 ng/µl with 1x FcRn Binding Buffer 2 (5 µl/well).
3. Add 5 µl of diluted enzyme to each well, except "Blank" wells.
4. Add 5 µl of 1x FcRn Binding Buffer 2 to the "Blank" wells.
5. Incubate at RT for 1 hour with gentle agitation.
6. Read the TR-FRET signal in a microtiter-plate reader under settings described below (settings may need optimization depending on the instrument).
7. The "Blank" value should be subtracted from all other values.

	Blank	**Positive Control**	**Test Compound**
Master Mixture	13 µl	13 µl	13 µl
1x FcRn Binding Buffer 2	5 µl	-	-
Test Compound	-	-	2 µl
Diluent Solution	2 µl	2 µl	-
Pre-incubate for 15 minutes at RT			
Diluted FcRn-Biotin (2.5 ng/µl)	-	5 µl	5 µl
Total	**20 µl**	**20 µl**	**20 µl**

Instrument Settings

Two sequential measurements should be conducted. Eu-donor emission should be measured at 620 nm followed by dye-acceptor emission at 665 nm. Data analysis is performed using the TR-FRET ratio (665 nm emission/620 nm emission).

Reading Mode	Time Resolved
Excitation Wavelength	320 (bandwidth 10 nm)
Emission Wavelength	620 (bandwidth 10 nm)
Lag Time	60 µs
Integration Time	500 µs
Excitation Wavelength	320 (bandwidth 10 nm)
Emission Wavelength	665 (bandwidth 10 nm)
Lag Time	60 µs
Integration Time	500 µs

CALCULATING RESULTS

Data analysis is performed using the TR-FRET ratio (665 nm emission/620 nm emission).

$$FRET = \frac{S_{665}}{S_{620}}$$

When percentage activity is calculated, the FRET value from the Blank (it is expected that Blank and Negative Control have a similar values) can be set as zero percent activity and the FRET value from the positive control can be set as one hundred percent activity.

$$\%\ Activity = \frac{FRET_S - FRET_{blank}}{FRET_P - FRET_{blank}} \times 100\%$$

$FRET_S$ =FRET value for samples of Test Inhibitor, $FRET_{blank}$ = FRET value for the Blank, and $FRET_p$ = FRET value for the Positive Control (no inhibitor).

Example Results

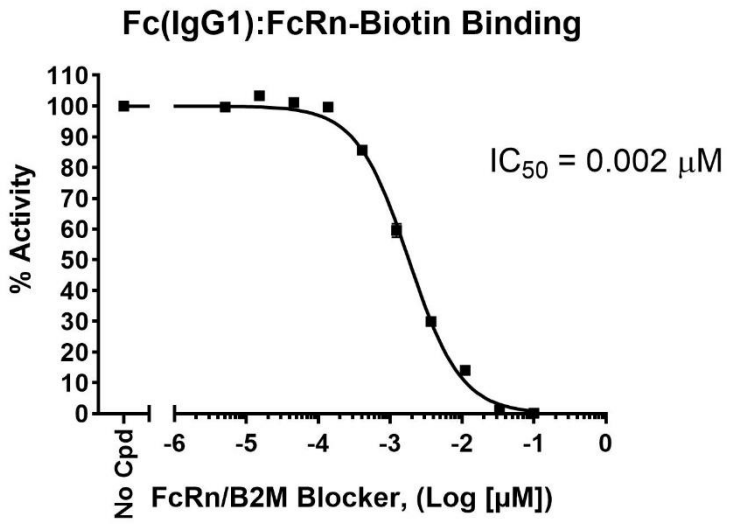

Figure 2. Inhibition of Fc (IgG1): FcRn binding by FcRn (FCGRT/B2M) Blocker.
Fc (IgG1): FcRn binding was evaluated in the presence of increasing concentrations of FcRn (FCGRT/B2M) Blocker (#101468). Results are expressed as percent activity, in which the binding activity in the absence of inhibitor is 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 (FCGRT/B2M) 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
FcRn: IgG Recycling HMEC-1 Cell Pool	82163	2 vials

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