Description

The FcRn: IgG Recycling HMEC-1 Cell Pool is a HMEC-1 cell pool expressing Fc gamma receptor and transporter (FCGRT) (NM_004107.5) and beta-2-microglobulin (B2M) (NM_004048.4), separated by a T2A self-cleaving peptide, under the control of a CMV promoter, introduced into HMEC-1 cells via lentiviral transduction. This cell pool is designed to measure the uptake and recycling of human IgG antibodies (human FcRn does not bind mouse IgG).

This cell pool has been validated with Nivolumab, Rituximab, Trastuzumab, anti-PCSK9 and an anti-IL-2Ra antibody.

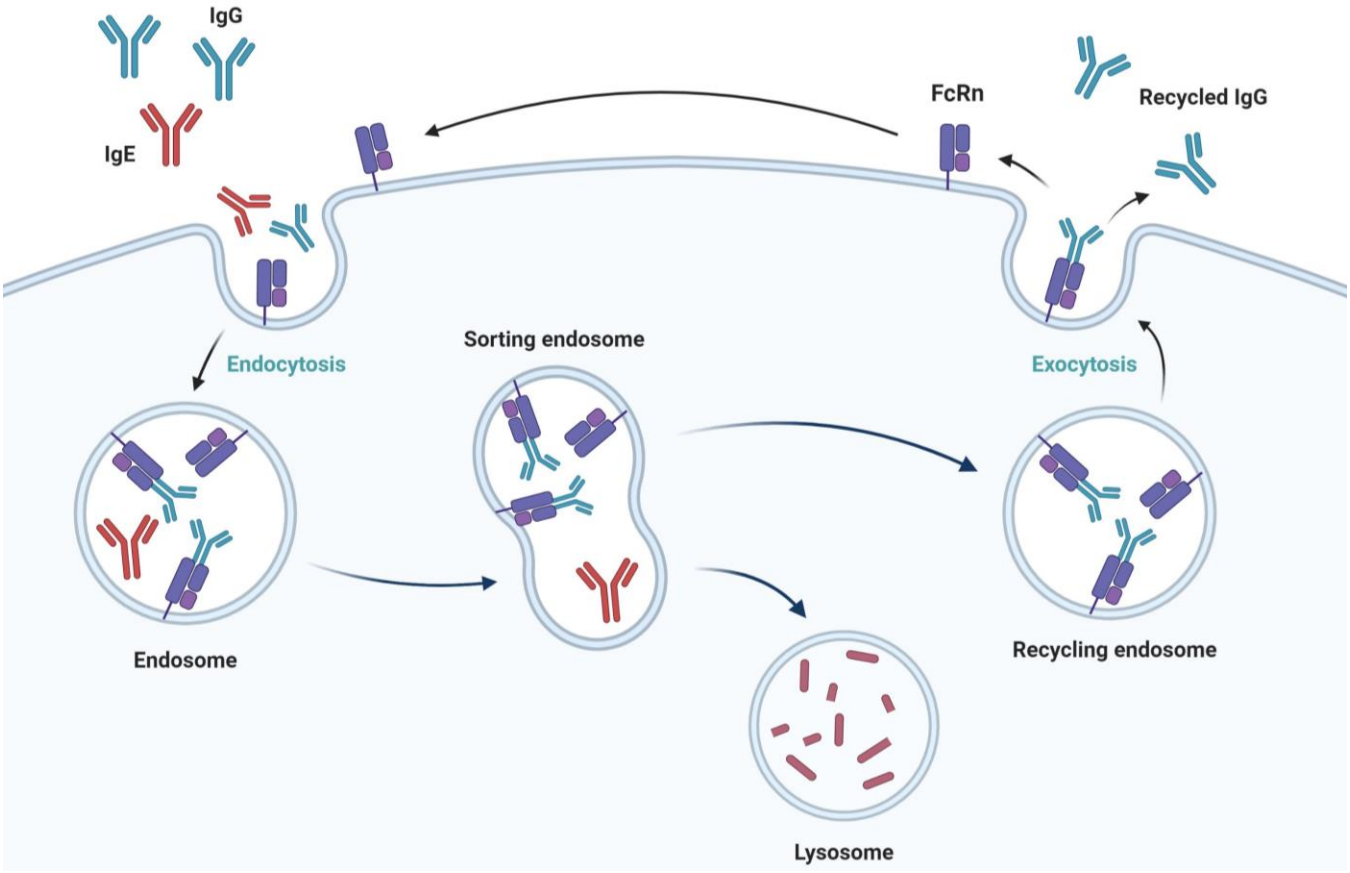


Image created with BioRender.com

Figure 1: Illustration of FcRn: IgG Recycling HMEC-1 Cell Pool mechanism of IgG FcRn-mediated recycling.
Cells are incubated with an antibody (IgG) of interest in a low pH Dilution Buffer, allowing FcRn to bind to IgG. Cells are then washed, and IgG recycling can be analyzed after 24 hours. The recycled antibody will be present in the cell culture media. If the antibody is retained inside the cells, detection can be done by lysing the cells and measuring the cellular content by ELISA or another method of interest.

Background

Neonatal Fc receptor for IgG (FcRn) is a heterodimeric protein. FcRn consists of Fc Gamma Receptor and Transporter, encoded by the FCGRT gene, and 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 the mother to the fetus. FcRn contributes to 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 plays a vital role in regulating the level of albumin and IgGs in circulation by binding albumin and the Fc region of IgG at low pH (about pH 6.0) in endosomes. It then diverts them from being degraded by lysosomes and instead recycles them for release into the neutral pH (about 7.0-7.4) of the extracellular compartment. The loss of FcRn in tumor cells has been reported to play a role in cancer by increasing the breakdown of albumin into amino acids needed for tumor cells proliferation. 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 thus an extended half-life (Evusheld) has been used to treat COVID-19. There are now several other drugs in clinical using similar strategies. Further studies and drug development taking advance of FcRn properties will provide new therapeutic options.

Application

- Determine profile of antibody recycling via FcRn.
- Screen for inhibitors of FcRn IgG recycling.

Materials Provided

Components	Format
2 vials of frozen cells	Each vial contains >1 x 10^6 cells in 1 ml of 7.5% DMSO and 92.5% FBS

Parental Cell Line

HMEC-1, Human dermal microvascular, endothelial-like cells, adherent

Mycoplasma Testing

The cell line has been screened to confirm the absence of Mycoplasma species.

Materials Required but Not Supplied

These materials are not supplied with the cell pool but are necessary for cell culture and cellular assays. BPS Bioscience's reagents are validated and optimized for use with this cell pool and are highly recommended for best results. Media components are provided in the Media Formulations section below.

Media and Supplements Required for Cell Culture

Name	Ordering Information
Thaw Medium 18	BPS Bioscience #82205
Growth Medium 18A	BPS Bioscience #82206
Assay Medium 8A	BPS Bioscience #82207
EGF Recombinant	BPS Bioscience #90201-3

Materials Used in the Cellular Assay

Name	Ordering Information
FcRn Recycling Wash Buffer (pH 7-7.4)	BPS Bioscience #82208
FcRn Recycling Dilution Buffer (pH 6)	BPS Bioscience #82209
Human IgG ELISA Kit	Novus #NBP3-00400
Nivolumab (Anti-PD-1 Antibody)	Selleckchem #A2002
Rituximab (Anti-CD20 Antibody)	Selleckchem #A2009
Trastuzumab (Anti-HER2 Antibody)	Selleckchem #A2007
Human Proprotein Convertase 9/PCSK9 Antibody	R&D Systems #MAB10347
Human CD25/IL-2Rα Antibody	R&D Systems #MAB9926
FcRn (FCGRT/B2M) Blocker	BPS Bioscience #101468
EGF Recombinant	BPS Bioscience #90201-3
PD-1, FLAG-Avi-His-Tag, Biotin-Labeled (Human) Recombinant	BPS Bioscience #71325
96 well plates	
Plate reader compatible with ELISA	

Storage Conditions

Cells are shipped in dry ice and should immediately be thawed or stored in liquid nitrogen upon receipt. Do not use a -80°C freezer for long term storage. Contact technical support at support@bpsbioscience.com if the cells are not frozen in dry ice upon arrival.

Media Formulations

For best results, the use of validated and optimized media from BPS Bioscience is *highly recommended*. Other preparations or formulations of media may result in suboptimal performance.

Note: Thaw Media do *not* contain selective antibiotics. However, Growth Media *do* contain selective antibiotics, which are used to maintain selective pressure on the cell population expressing the gene of interest. Cells should be grown at 37°C with 5% CO_2. BPS Bioscience's cell lines are stable for at least 10 passages when grown under proper conditions.

Media Required for Cell Culture

Thaw Medium 18 (BPS Bioscience #82205):

MCDB131 medium supplemented with 10% FBS, 10 mM Glutamine, 1 µg/ml Hydrocortisone, 1% Penicillin/Streptomycin.

Prepare Thaw FcRn: IgG Recycling HMEC-1 Cell Pool Medium: Thaw Medium 18 + 10 ng/ml Human Epidermal Growth Factor (BPS Bioscience #90201-3)

Growth Medium 18A (BPS Bioscience #82206):

MCDB131 medium supplemented with 10% FBS, 10 mM Glutamine, 1 µg/ml Hydrocortisone, 1% Penicillin/Streptomycin and 500 ng/ml Puromycin.

Prepare Growth FcRn: IgG Recycling HMEC-1 Cell Pool Medium: Growth Medium 18A + 10 ng/ml Human Epidermal Growth Factor (BPS Bioscience #90201-3)

Media Required for Functional Cellular Assay

Thaw FcRn: IgG Recycling HMEC-1 Cell Pool Medium:
Thaw Medium 18 + 10 ng/ml Human Epidermal Growth Factor (BPS Bioscience #90201-3).

Assay Medium 8A (BPS Bioscience #82207):
MCDB131 medium and 1% Penicillin/Streptomycin.

Cell Culture Protocol

Cell Thawing

1. Swirl the vial of frozen cells for approximately 60 seconds in a 37°C water bath. As soon as the cells are thawed (it may be slightly faster or slower than 60 seconds), quickly transfer the entire contents of the vial to a tube containing 10 ml of pre-warmed Thaw FcRn: IgG Recycling HMEC-1 Cell Pool Medium.

   Note: Leaving the cells in the water bath at 37°C for too long will result in rapid loss of viability.

2. Immediately spin down the cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in 5 ml of pre-warmed Thaw FcRn: IgG Recycling HMEC-1 Cell Pool Medium.

3. Transfer the resuspended cells to a T75 flask and incubate at 37°C in a 5% CO_2 incubator.

4. After 24 hours of culture, check for cell attachment and viability. Change medium to fresh Thaw FcRn: IgG Recycling HMEC-1 Cell Pool Medium and continue growing in a 5% CO_2 incubator at 37°C until the cells are ready to passage.

5. Cells should be passaged before they are fully confluent. At first passage and subsequent passages, use Growth FcRn: IgG Recycling HMEC-1 Cell Pool Medium.

Cell Passage

HMEC-1 cell growth slows down after roughly 6 passages, resulting in a higher doubling time and lower cell yield per flask. It is recommended that stock vials are frozen soon after the first passage. At first thaw the yield is expected to be around 7 million cells per T75 flask. This decreases to around 2-3 million cells at passages 4-5 and then to about 1.5 million cells after passage 7.

1. When most of the surface area of the flask is covered with cells, aspirate the medium, wash cells with phosphate buffered saline (PBS) without Ca^{2+}/Mg^{2+} twice.

2. Wash the cells once with 0.05% Trypsin/EDTA.

3. Add 0.05% Trypsin/EDTA and allow cells to detach from the culture vessel with. Pipet up and down until a single cell suspension is obtained (check under the microscope).

4. Once the cells have detached, add 10 ml of Growth FcRn: IgG Recycling HMEC-1 Cell Pool Medium and transfer to a tube.

5. Spin down the cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in Growth FcRn: IgG Recycling HMEC-1 Cell Pool Medium.

6. Seed into new culture vessels at the recommended sub-cultivation ratio of 1:5 twice a week.

   Note: Decrease split ratio as needed once cells reach passage 6 and up.

Cell Freezing

1. Aspirate the medium, wash the cells with PBS without Ca^{2+}/Mg^{2+}, and detach the cells from the culture vessel with 0.05% Trypsin/EDTA.

2. Once the cells have detached, add Growth FcRn: IgG Recycling HMEC-1 Cell Pool Medium and count the cells.

3. Spin down the cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in HMEC1 Freezing Media (7.5% DMSO and 92.5% FBS) at ~2 x 10^6 cells/ml.

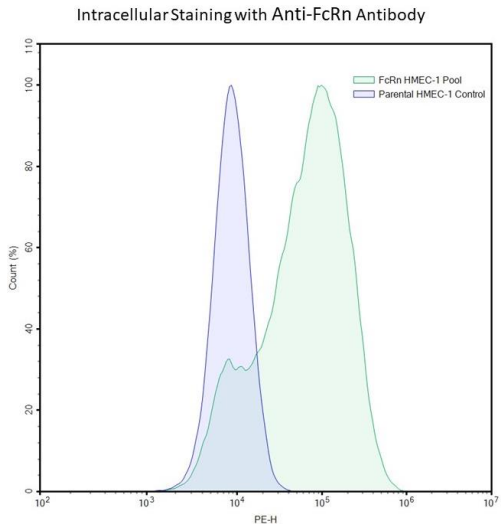
4. Dispense 1 ml of cell suspension into each cryogenic vial. Place the vials in an insulated container for slow cooling and store at -80°C overnight.

5. Transfer the vials to liquid nitrogen the next day for storage.

Note: It is recommended to expand the cells and freeze at least 10 vials at an early passage for future use.

A. Validation Data

a.



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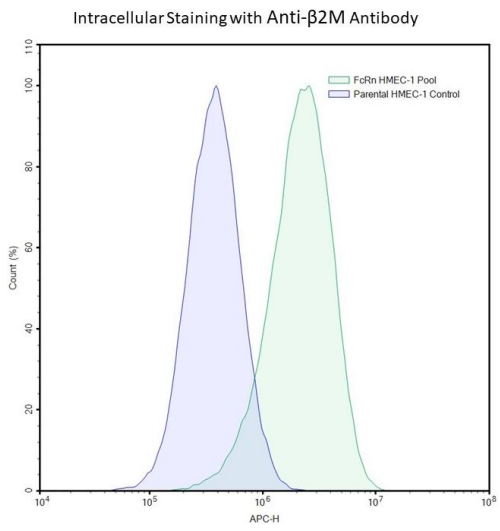

Figure 2: Analysis of FCGRT and B2M expression in FcRn: IgG Recycling HMEC-1 Cell Pool by flow cytometry.
Parental HMEC-1 cells (blue) and FcRn HMEC-1 cells (green) were fixed with Fixation Buffer (BioLegend #420801) and stained intracellularly. (a) FCGRT expression was detected with Human FcRn Antibody (R&D Systems #MAB8639) followed by PE Goat anti-mouse IgG (minimal x-reactivity) Antibody (BioLegend #405307). (b) For B2M expression, cells were stained with APC anti-human β2-microglubulin Antibody (BioLegend #316312). The Y-axis represents the cell count. The X-axis indicates the fluorophore intensity.

Functional Assay

A. pH dependent recycling of antibodies of interest in the FcRn: IgG Recycling HMEC-1 Cell Pool

- The following assay is designed for a 96-well format. To perform the assay in different tissue culture formats, the cell number and reagent volumes should be scaled appropriately.
- The experiments should be performed in triplicate.
- The assay should include "No Antibody Control" and "Test Compound" conditions.
- It is recommended that a standard curve of the test antibody is performed to ensure detection by Human IgG ELISA Kit (Novus #NBP3-00400), or other assay of your choice.

Day 1:

1. Seed FcRn: IgG Recycling HMEC-1 cells at a density of 30,000 cells/well in 100 µl of Thaw FcRn: IgG Recycling HMEC-1 Cell Pool Medium into a 96-well plate.

2. Incubate the cells at 37°C in a 5% CO_2 incubator for 48 hours.

Day 2:

1. Incubate FcRn Recycling Wash Buffer at 4°C (to be used at later steps) (you will need 4 x 100 µl/well).

   Note: Leave enough FcRn Recycling Wash Buffer at Room Temperature (RT) for a separate wash step (you will need 100 µl/well).

Day 3:

1. Remove spent media from the cells.

2. Add 100 µl/well of RT FcRn Recycling Wash Buffer.

3. Incubate the cells at 37°C in a 5% CO_2 incubator for 1 hour.

4. Prepare a dilution of the test antibody(s) in FcRn Recycling Dilution Buffer (100 µl/well).

5. Remove FcRn Recycling Wash Buffer from cells.

6. Add 100 µl of diluted antibody to the "Test Compound" wells.

7. Add 100 µl of the FcRn Recycling Dilution Buffer to the "No Antibody Control".

8. Incubate the cells at 37°C in a 5% CO_2 incubator for 4 hours.

9. Remove solution from the cells and wash 4 times with ice cold FcRn Recycling Wash Buffer (incubated at 4°C).

10. Add 100 µl of Assay Medium 8A to all wells.

11. Incubate 37°C in a 5% CO_2 incubator for 24 hours.

Day 4:

1. Transfer 90-100 µl of media from each well to a new 96-well plate.

   Note: The samples can be tested the same day or frozen at -80°C and used within a week. When freezing the samples, seal the plates with a plastic plate sealer, cover, and place in the freezer. When ready to use, thaw plates at 4°C and continue with the protocol. The remaining cells can be discarded or lysed with an appropriate lysis buffer and tested to determine the amount of antibody retained inside the cells.

2. Spin down the plate at 450 *x g* for 5 minutes to pellet any cell debris.

3. Transfer 90 µl of media to each well of the IgG ELISA plate.

4. Prepare a standard curve of the test antibody.

5. Perform ELISA following the manufacturer's protocol.

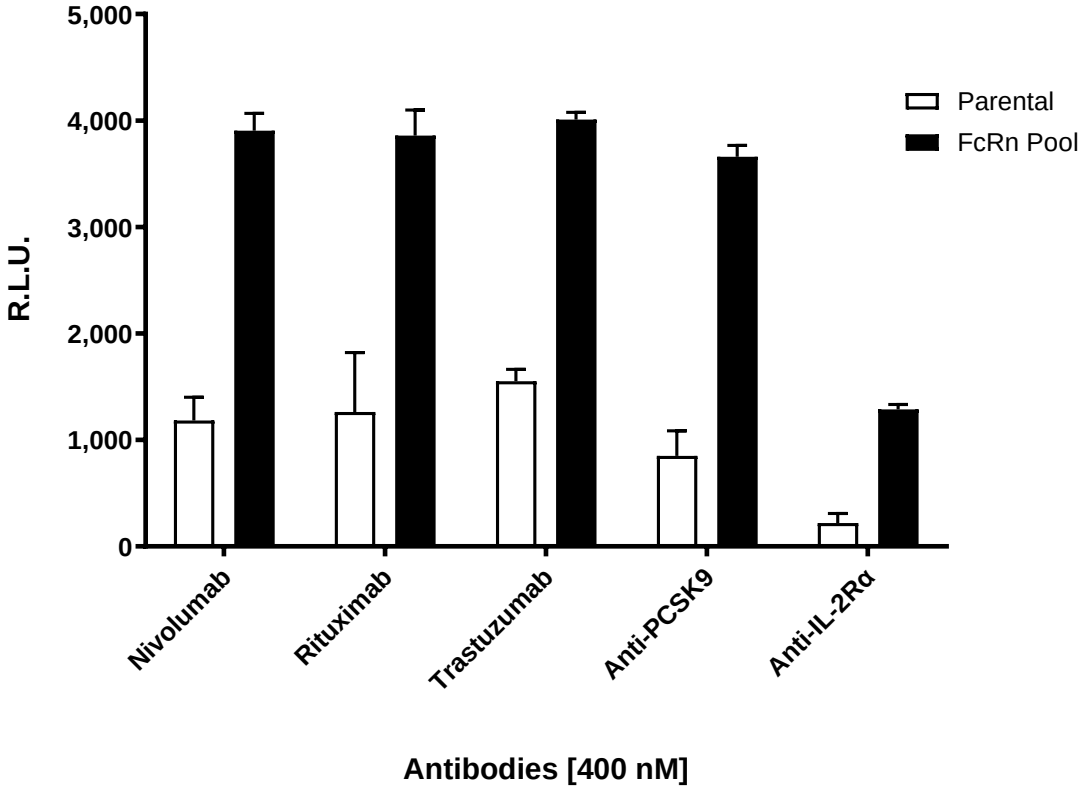

Figure 3: pH dependent antibody recycling in FcRn: IgG Recycling HMEC-1 Cell Pool and parental HMEC-1 cells.
Nivolumab, Rituximab, Trastuzumab, anti-PSCK9 and anti-IL2-Rα antibodies were prepared in FcRn Recycling Dilution Buffer (pH 6) at 400 nM and added to the cells for 4 hours. The low pH of the FcRn Recycling Dilution Buffer facilitated the binding of the antibodies to FcRn. Cells were washed, warm assay medium was added, and cells were incubated for 24 hours to allow for IgG recycling. The sample medium was collected and analyzed by ELISA. Results are shown as relative light units (RLU).

B. Inhibition of pH dependent FcRn IgG recycling by an FcRn blocker in FcRn: IgG Recycling HMEC-1 Cell Pool

- The following assay is designed for a 96-well format. To perform the assay in different tissue culture formats, the cell number and reagent volumes should be scaled appropriately.
- The experiments should be performed in triplicate.
- The assay should include "No Inhibitor Control", 'No Antibody Control" and "Test Compound" conditions.
- It is recommended that a standard curve of the test antibody is performed to ensure detection by Human IgG ELISA Kit (Novus #NBP3-00400), or other assay of your choice.
- If using FcRn Blocker (BPS Bioscience #101468), the use of the Fc biotin-labeled detection agent contained in the IgG ELISA plate (Novus #NBP3-00400) is not recommended and can impact the results. It is recommended to use an alternative biotinylated antigen specifically targeting your antibody of interest (versus an Fc targeting antibody). For example, if using human PD-1 biotin-labeled protein to detect Nivolumab, it is recommended to use an anti-PD-1 antibody for detection.

Day 1:

1. Seed FcRn: IgG Recycling HMEC-1 cells at a density of 30,000 cells/well in 100 µl of Thaw FcRn: IgG Recycling HMEC-1 Cell Pool Medium into a 96-well plate.

2. Incubate the cells at 37°C in a 5% CO_2 incubator for 48 hours.

Day 2:

1. Incubate FcRn Recycling Wash Buffer at 4°C (to be used at later steps) (you will need 4x 100 µl/well).

   Note: Leave enough FcRn Recycling Wash Buffer at RT for a separate wash step (you will need 100 µl/well).

Day 3:

1. Prepare FcRn blocker dilutions in FcRn Recycling Wash Buffer (100 µl/well).

2. Remove spent media from cells.

3. Add 100 µl of diluted FcRn Blocker to the "Test Compound" wells.

4. Add 100 µl of FcRn Recycling Wash Buffer to the "No Inhibitor Control" and "No Antibody Control" wells.

5. Incubate the cells at 37°C in a 5% CO_2 incubator for 1 hour.

6. Prepare a dilution of the test antibody(s) of interest in FcRn Recycling Dilution Buffer at a concentrations 2-fold higher than final desired concentration (50 µl/well).

7. Prepare additional FcRn Blocker at concentrations 2-fold higher than final desired concentration in FcRn Recycling Dilution Buffer (50 µl/well).

8. After the 1 hour incubation, remove the solution from cells.

9. Add 50 µl of diluted test antibody to the "Test Compound" and "No Inhibitor Control" wells.

10. Add 50 µl of FcRn Blocker to the "Test Compound" wells.

11. Add 50 µl of FcRn Recycling Dilution Buffer to the "No Inhibitor Control" wells.

12. Add 100 µl of FcRn Recycling Dilution Buffer to the "No Antibody Control" wells.

13. Incubate the cells at 37°C in a 5% CO_2 incubator for 4 hours.

14. Remove FcRn Recycling Dilution Buffer from the cells and wash 4 times with ice cold Wash Buffer (incubated at 4°C).

15. Add 100 µl of Assay Medium 8A to all wells.

16. Incubate 37°C in a 5% CO_2 incubator for 24 hours.

Day 4:

1. Transfer 90-100 µl of media from each well to a new 96-well plate.

   Note: The samples can be tested the same day or frozen at -80°C and used within a week. When freezing the samples, seal the plates with a plastic plate sealer, cover, and place in freezer. When ready to use, thaw plates at 4°C and continue with the protocol. The remaining cells can be discarded or lysed with an appropriate lysis buffer and tested to determine the amount of antibody retained inside the cells.

2. Spin down the plate at 450 *x g* for 5 minutes to pellet any cell debris.

3. Transfer 90 µl of media to each well of the IgG ELISA plate.

4. Prepare a standard curve of the test antibody.

5. Perform ELISA following the manufacturer's protocol.

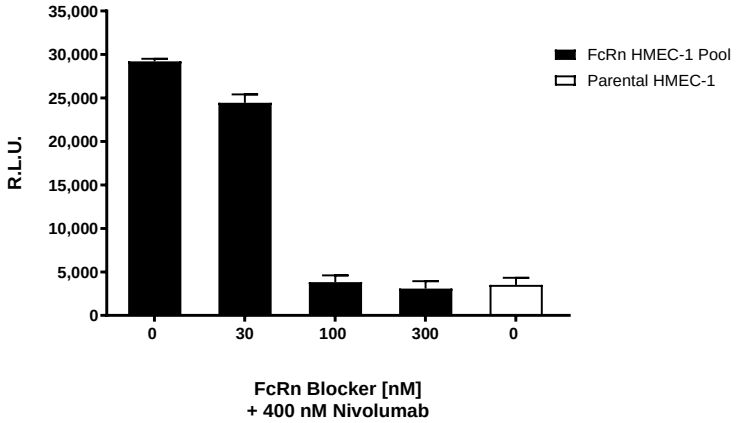

Figure 4: Inhibition of FcRn recycling activity in the FcRn: IgG Recycling HMEC-1 Cell Pool using a FcRn Blocker.
FcRn Blocker prepared in Wash Buffer was added to the cells for one hour. After the 1 hour incubation, FcRn Blocker dilutions and Nivolumab (400 nM) were prepared in FcRn Recycling Dilution Buffer (pH 6) at concentrations 2-fold higher than the desired final concentration. After addition cells were incubated for 4 hours. Cells were then washed and incubated for an additional 24 hours. The sample medium was collected and analyzed by ELISA. Results are shown as relative light units (RLU).

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.

References

Grevys A, *et al.,* 2018 *Nature Communications,* 9: 621.
Swiercz R, *et al.,* 2017 *Oncotarget,* 8(2): 3528–3541.
Weflen A, *et al.,* 2013 *Mol Biol Cell,* 24(15): 2398–2405.

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Troubleshooting Guide

Visit bpsbioscience.com/cell-line-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com.

Related Products

Products	*Catalog #*	*Size*
Fc (IgG1): FcRn Inhibitor Screening Colorimetric Assay Kit	78501	96 reactions
HSA:FcRn Small Molecule Screening Chemiluminescence Assay Kit	82152	96 reactions
FcRn (FCGRT/B2M), His-Tag Recombinant	71285-1	100 µg
FcRn Complex (FCGRT/B2M), His-Avi-Tag, Biotin-Labeled, HiP™ Recombinant	71283-1	25 µg
FcRn (FCGRT/B2M), His-Tag (Mouse) HiP™ Recombinant	11349-1	25 µg
Anti-CD20 Functional Antibody	71209	100 µg

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