Description

The IgG Colorimetric ELISA Kit is designed to detect and quantify protein levels of human IgG captured by pre-matched antibody pairs. This assay kit comes in a convenient 96-well format, with a pre-coated IgG-specific antibody plate, enough recombinant purified IgG1 standard, and detection reagents for 100 wells.

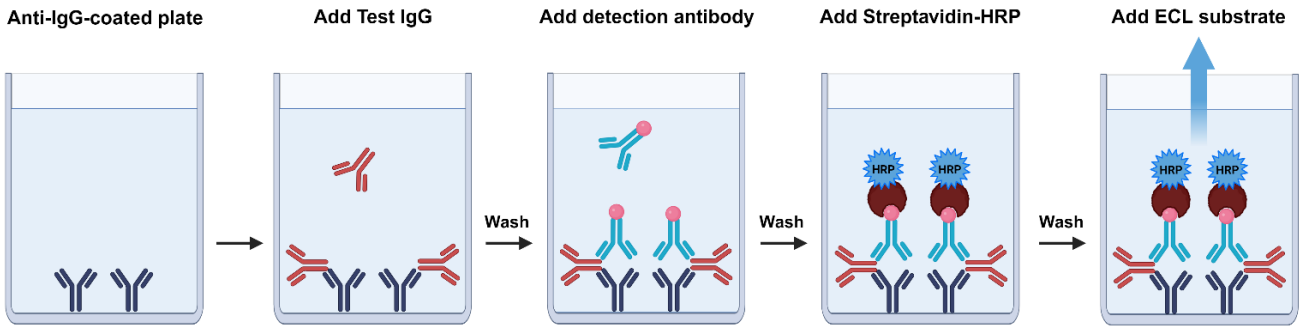

Figure 1: Human IgG Colorimetric ELISA Kit assay principle.

Background

IgG, or immunoglobulin G, is one of the most abundant glycoproteins present in human serum. Humans have four different IgG subclasses, IgG1-4, where the number corresponds to their abundance. IgG are involved in complement activation, phagocytosis, and toxin neutralization. Each subclass binds to different FcγR receptors, with varying affinities. Out of the four isotypes, IgG1 accounts for 65% of all IgG and has high affinity for most of the FcγRs, but its affinity for FcRn (neonatal Fc receptor for IgG) is pH dependent (higher at pH 6.5 or below). The binding 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 advantage of FcRn properties will provide new therapeutic options. The ability to accurately detect human IgG levels with an easy readout is thus a necessary tool to advance the development of such therapeutic tools.

Applications

Quantify human IgG in cell culture supernatants.

Supplied Materials

Catalog #	Name	Amount	Storage
	96-well Anti-IgG Pre-Coated Plate	1	+4°C
82767	Anti-IgG Detection Antibody	6 µl	-80°C
82724	Streptavidin HRP	6 µl	-80°C
79743	Blocking Buffer 3	25 ml	+4°C
71456	IgG1 Standard*	2 µg	-80°C
79651	HRP Colorimetric Substrate	10 ml	+4°C
	Adhesive plate seal	1	Room Temperature

**The concentration of the protein is lot-specific and will be indicated on the tube.*

Materials Required but Not Supplied

- Test Samples
- 1x PBS (Phosphate Buffer Saline)
- PBST Buffer (1x PBS with 0.05% Tween-20)
- 2 M sulfuric acid (aqueous)
- Diluent Solution (e.g. cell culture medium like DMEM (Dulbecco's Modified Eagle Medium))
- UV/Vis spectrophotometer microplate reader capable of reading absorbance at 450 nm*
- Adjustable micropipettor and sterile tips
- Orbital shaker

**Alternately, a spectrophotometer reading at 650 nm may be used, but sensitivity of the assay will be greatly reduced.*

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

The IgG Colorimetric ELISA Kit is compatible with up to 1% final DMSO concentration.

Assay Protocol

- All samples and controls should be performed in duplicate.
- The assay should include “Negative Control”, “IgG Standard”, and “Test Sample” conditions.
- We recommend maintaining the diluted antibody on ice during use.
- Variation in sample collection, processing and storage may cause differences in sample assay results.
- We recommend using IgG1 Standard (#71456) as internal control. If not running a full standard curve, we recommend running the IgG standard at 0.1X, 1X and 10X the EC_{50} value shown in the validation data below.
- The standard provided with this assay kit consists of purified IgG1 only. If other IgG isotypes are analyzed, we recommend using an alternative standard related to the IgG isotype to be analyzed, for accurate quantitative comparison.
- For detailed information on protein handling please refer to Protein FAQs (bpsbioscience.com).
- We recommend adding protease inhibitors (#82199) to samples and store samples at -80°C to avoid loss of bioactive human IgG.
- Samples containing a visible precipitate must be clarified prior to use in the assay.
- Avoid repeated freeze-thaw cycles. The frozen sample should be thawed on ice and mixed gently.
- The linear range of the assay is: 0 -25 ng/ml.

Step 1: IgG Binding

1. Rehydrate the plate by adding 200 µl of PBST to every well.
2. Incubate 15 minutes at Room Temperature (RT).
3. Remove the PBST and tap the plate onto clean paper towels to remove all liquid.
4. Thaw the IgG Standard on ice. Briefly spin the tube to recover the full content of the tube.
5. Dilute the IgG Standard to 2 ng/µl (50 µl/well) in the same diluent solution that was used in sample preparation. This will correspond to the highest value on the standard curve.

   Note: It is recommended to use the same buffer or medium as Diluent Solution as the one used in the preparation of the "Test Sample". For example, if the uptake and recycling of human IgG antibodies by a pool of cells is analyzed, the same cell culture medium should be used as a Diluent Solution to prepare an IgG standard. Alternatively, PBS or PP-02 Buffer (#82620, sold separately) can be used.
6. Prepare a serial dilution (1:3 recommended) of the diluted IgG Standard using the preferred Diluent Solution (50 µl/well).
7. Add 50 µl of Diluent Solution to “Negative Control” wells.
8. Add 50 µl of IgG Standard dilutions to wells labeled “IgG Standard”.
9. Add 50 µl of each test sample to wells labeled "Test Sample”.
10. Incubate the plate at RT with slow agitation for 1 hour.
11. Wash the plate three times with 200 µl of PBST Buffer per well.

Step 2: Detection

1. Dilute Anti-IgG Detection Antibody 1000-fold in Blocking Buffer 3 (50 µl/well).
2. Add 50 µl to each well.
3. Incubate at RT with shaking for 45 minutes to 1 hour.
4. Wash plate three times with 200 µl of PBST Buffer per well.
5. Dilute Streptavidin HRP 1000-fold in Blocking Buffer 3 (50 µl/well).
6. Add 50 µl to each well.
7. Incubate at RT with shaking for 30 minutes.

8. Wash plate three times with 200 µl of PBST Buffer per well.

9. Add 100 µl of the colorimetric HRP substrate to each well.

10. Incubate the plate at the RT until blue color is developed in the "Positive Control" wells.

    Note: For IgG, it normally takes 10-15 minutes to fully develop the color. However, the optimal incubation time may vary, and should be determined empirically by the user.

11. Add 100 µl of 2 M sulfuric acid to each well.

12. Read the absorbance at 450 nm using a UV/Vis spectrophotometer microplate reader.

13. The "Negative Control" value should be subtracted from all other values.

14. If applicable generate a standard curve of absorbance versus IgG standard concentrations and determine concentration of the "Test sample". For detailed information regarding standard curve and determination of the "Test sample" concentration refer to https://bpsbioscience.com/assay-kits-faq.

    Note: The "Negative Control" absorbance value should be ~0.05 at 450 nm. Alternatively, the plate may be read at 650 nm without adding 2 M sulfuric acid, but the Signal-to-Background ratio will be decreased.

Example Results

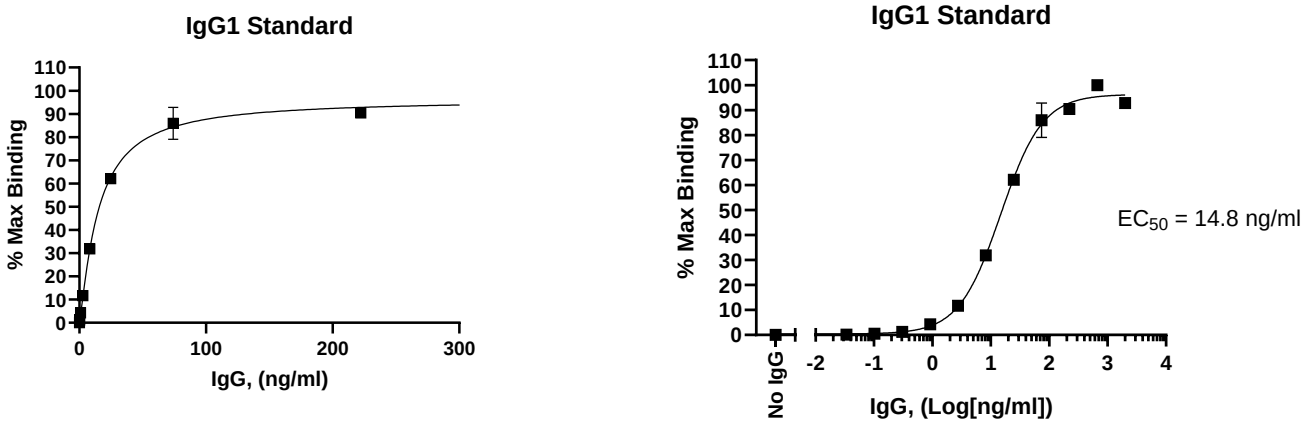

Figure 2. Example of IgG standard curves.
Various amounts of the IgG1 standard were run in duplicate.

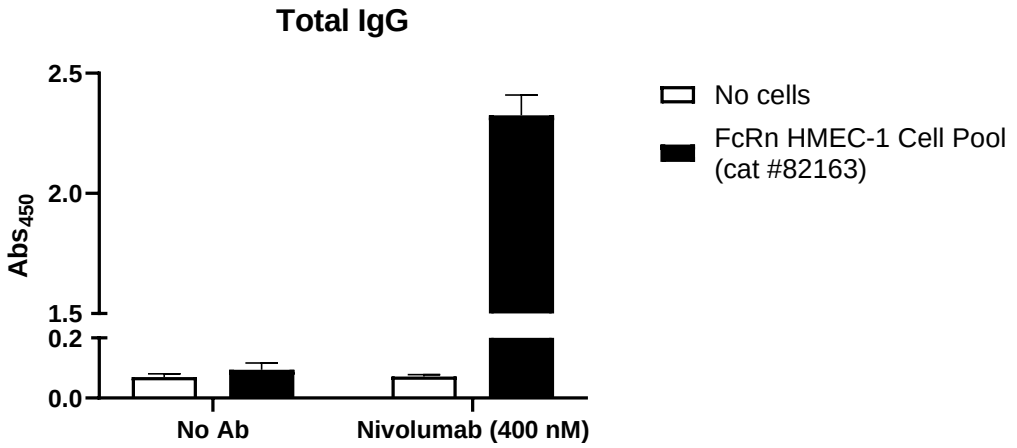

Figure 2. Example of IgG recycling quantification in FcRn HMEC-1 Cell Pool treated with Nivolumab.
Nivolumab was prepared in FcRn Recycling Dilution Buffer (pH 6) (#82209) at 400 nM and added to FcRn: IgG Recycling HMEC-1 Cell Pool (#82163) for 4 hours. Cells were washed, warm MCDB 131 medium plus 1% Penicillin/Streptomycin medium (Assay Medium 8A, #82207) was added, and cells were incubated for 24 hours to allow for IgG recycling. The sample medium was collected and analyzed. Results are shown as raw luminescence readings.

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

References

Vidarsoon G, *et al.*, 2014 *Front. Immunol.* 5:00520.

Troubleshooting Guide

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

Related Products

Products	*Catalog #*	*Size*
Human IgG Chemiluminescence ELISA Kit	82611	96 reactions
FcRn: IgG Recycling HMEC-1 Cell Pool	82163	2 vials
FcRn Recycling Wash Buffer (pH7-7.4)	82208	75 ml
FcRn Recycling Dilution Buffer (pH6)	82209	15 ml
FcRn (FCGRT/B2M) Blocker	101468	50 µg/100 µg
IgG4, Fc (Human) HiP™ Recombinant	71457	200 µg
Anti-Human IgG, Unconjugated Antibody	100736	100 µg/500 µg

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