Rat IgG Immunoassay

Catalog Number: SEKR-0020

For the quantitative determination of rat IgG concentrations in cell culture supernates, serum, and plasma.

For research use only. Not for use in diagnostic procedures.

MANUFACTURED AND DISTRIBUTED BY:

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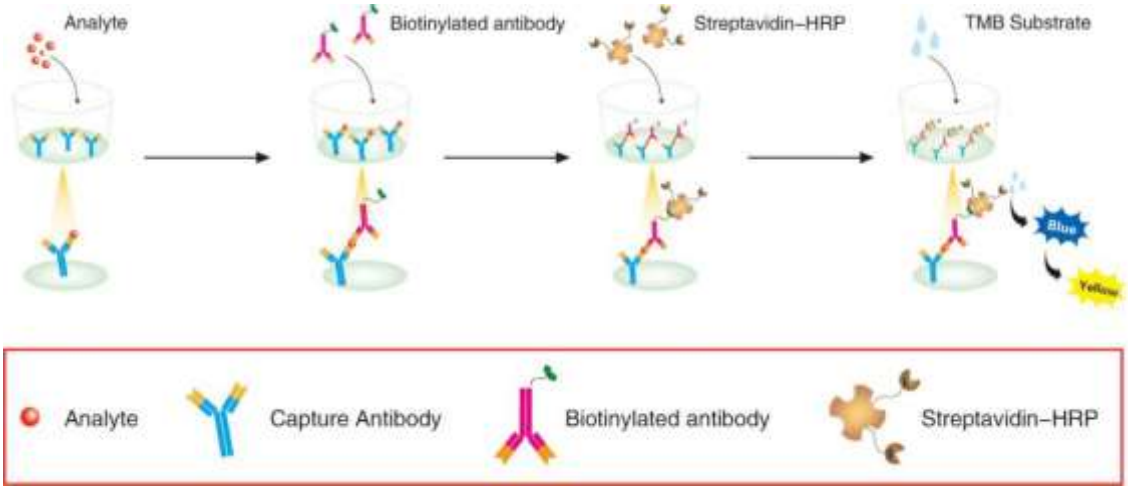
BACKGROUND

Immunoglobulin G (IgG) is antibody molecule. Each IgG is composed of four peptide chains — two heavy chains γ and two light chains. IgG antibodies are large molecules of about 150 kDa composed of four peptide chains. It contains two identical class γ heavy chains of about 50 kDa and two identical light chains of about 25 kDa, thus a tetrameric quaternary structure. The two heavy chains are linked to each other and to a light chain each by disulfide bonds. The resulting tetramer has two identical halves, which together form the Y-like shape. Each end of the fork contains an identical antigen binding site. The Fc regions of IgGs bear a highly conserved N-glycosylation site. The N-glycans attached to this site are predominantly corefucosylated diantennary structures of the complex type. In addition, small amounts of these Nglycans also bear bisecting GlcNAc and α-2, 6-linked sialic acid residues . IgG antibodies are involved in predominantly the secondary immune response. The presence of specific IgG, in general, corresponds to maturation of the antibody response . IgG can bind to many kinds of pathogens, for example viruses, bacteria, and fungi, and protects the body against them by agglutination and immobilization, complement activation (classical pathway), opsonization for phagocytosis, and neutralization of their toxins.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for IgG has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IgG present is captured by the coated antibody after incubation. Following extensive washing, a biotin-conjugate antibody specific for IgG is added to detect the captured IgG protein in sample. For signal development, horseradish peroxidase (HRP)-conjugated Streptavidin is added, followed by tetramethyl-benzidine (TMB) reagent. Following a wash to remove any unbound combination, and enzyme conjugate is added to the wells. Solution containing sulfuric acid is used to stop color development and the color intensity which is proportional to the quantity of bound protein is measurable at 450nm.

Schematic diagram：



1. This Solarbio ELISA should not be used beyond the expiration data on the kit label.
2. To avoid cross-contamination, use a fresh reagent reservoir and pipette tips for each step.
3. To ensure accurate results, some details, such as technique, plasticware and water sources should be emphasized.
4. A thorough and consistent wash technique is essential for proper assay performance.
5. A standard curve should be generated for each set of samples assayed.
6. It is recommended that all standards and samples be assayed in duplicate.
7. Avoid microbial contamination of reagents and buffers. Buffers containing protein should be made under aseptic conditions and be prepared fresh daily.
8. In order to ensure the accuracy of the results, the standard curve should be made every time.

PRECAUTIONS

The Stop Solution suggested for use with this kit is an acid solution. Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

KIT COMPONENTS& STORAGE CONDITIONS

PART	SIZE	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Microwell Plate - antibody coated 96-well Microplate (8 wells ×12 strips)	1 plate	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2 – 8°C**
Standard - lyophilized,100 ng/vial upon reconstitution	2 vials	Aliquot and Store at -20°C** for six months
Pre-titrated,HRP-conjugated anti-rat Ig(H+L) monoclonal antibody (100X) - 120 ul/vial	1 vial	Store at 2-8°C **for six months
Standard /Sample Diluent - 16 ml/vial	1 bottle	Store at 2-8°C** for six months
HRP-conjugated Diluent - 16 ml/vial	1 bottle	Store at 2-8°C** for six months
Wash Buffer Concentrate (20x) - 30 ml/vial	1 bottle	Store at 2-8°C** for six months
Substrate Solution - 12 ml/vial	1 bottle	Store at 2-8°C** for six months
Stop Solution - 12 ml/vial	1 bottle	Store at 2-8°C** for six months
Plate Cover Seals	4 pieces	

**Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED BUT NOT SUPPLIED

1. Microplate reader capable of measuring absorbance at 450 nm.
2. Pipettes and pipette tips.
3. Deionized or distilled water.
4. Squirt bottle, manifold dispenser, or automated microplate washer.
5. 500 mL graduated cylinder.

SPECIMEN COLLECTION & STORAGE

Cell Culture Supernates - Centrifuge cell culture media at 1000×g to remove debris. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

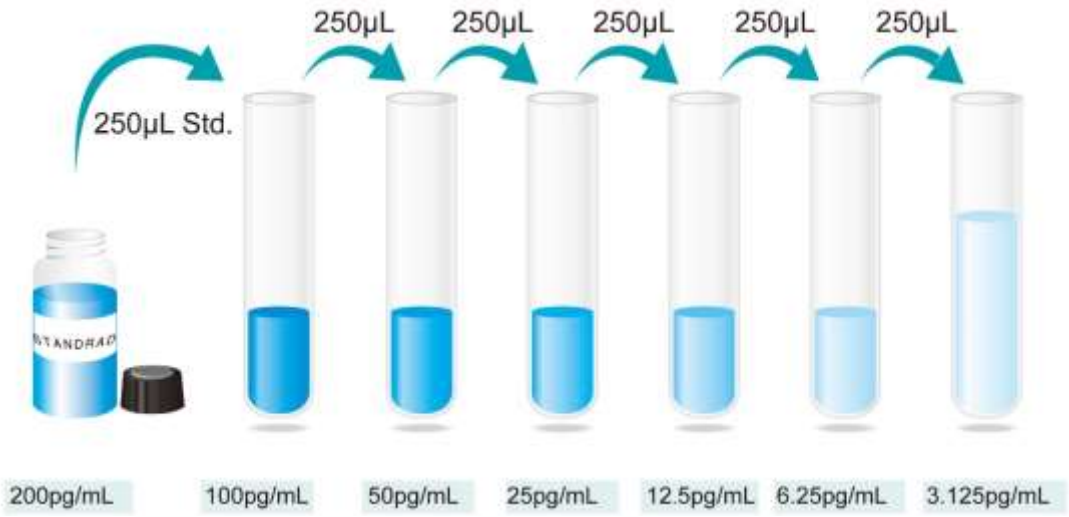
Serum - Use a serum separator tube (SST) and allow samples to clot for 2 hours at room temperature or overnight at 2-8℃. Centrifuge approximately for 15 minutes at 1000×g. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000×g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: The normal rat serum or plasma samples are suggested to make a 1:2 dilution.

REAGENTS PREPARATION

1. **Temperature returning** - Bring all kit components and specimen to room temperature (20-25°C) before use.
2. **Wash Buffer** - Dilute 30mL of Wash Buffer Concentrate with 570mL of deionized or distilled water to prepare 600mL of Wash Buffer. If crystals have formed in the concentrate Wash Buffer, warm to room temperature and mix gently until the crystals have completely dissolved.
3. **Standard/Sample** - Reconstitute the Standard with 570uL of Standard/Sample Diluent. This reconstitution produces a stock solution of 200ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 500µL of Standard/Sample Diluent into100ng/ml tube and the remaining tubes. Use the stock solution of 200ng/mL to produce a 2-fold dilution series (below). Mix each tube thoroughly and change pipette tips between each transfer. The 200 ng/mL standard serves as the high standard. The Standard/ Sample Diluent serves as the zero standard (0 pg/mL).



Preparation of IgG standard dilutions

***If you do not run out of re-melting standard, store it at -20℃. Diluted standard shall not be reused.**

4. **Working solution of HRP-Conjugate anti-rat IgG antibody:** Make a 1:100 dilution of the concentrated **HRP-conjugated anti-rat IgG monoclonal antibody** solution with the **HRP-conjugated** Diluent in a clean plastic tube.

   ***The working solution should be used within one day after dilution.**

ASSAY PROCEDURE

Prepare all reagents and standards as directed. Wash the plate 3 times before assay.

⇩

Add 100μl standard or samples to each well, incubate 120 minutes in room temperature.

⇩ Aspirate and wash 4 times

Add 100μl working solution of **HRP-conjugated anti-rat IgG antibody** to each well, incubate 60 minutes in room temperature.

⇩ Aspirate and wash 5 times

Add 100μl Substrate solution to each well, incubate 15-30 minutes,.Protect from light in room temperature.

⇩

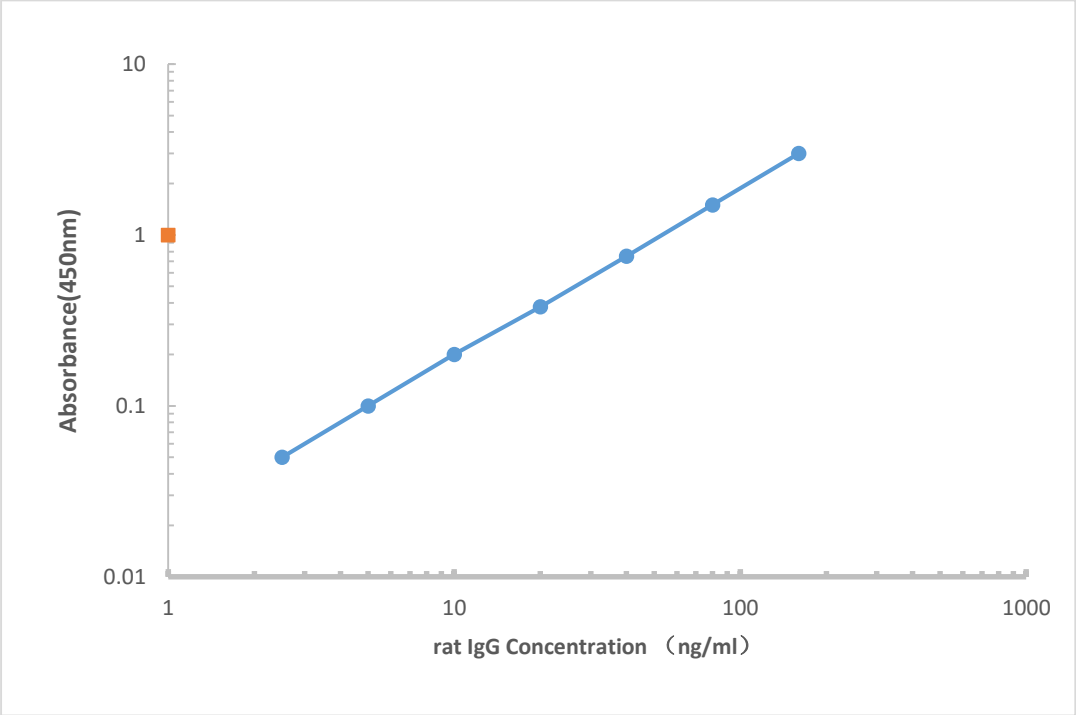
Add 50μl Stop solution to each well. Read at 450nm within 5 minutes.

CALCULATION OF RESULTS

1. The standard curve is used to determine the amount of specimens.
2. First, average the duplicate readings for each standard, control, and sample. All O.D. values are subtracted by the mean value of blank control before result interpretation.
3. Construct a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph.
4. The data may be linearized by plotting the log of the IgG concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.
5. This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Typical data using the IgG ELISA

Standard(ng/ml)	OD.	OD.	Average	Corrected
0	0. 052	0. 053	0. 052	———
3. 12	0. 086	0. 09	0. 088	0. 035
6. 25	0. 157	0. 171	0. 164	0. 111
12. 5	0. 269	0. 253	0. 261	0. 208
25	0. 426	0. 441	0. 433	0. 381
50	0. 773	0. 756	0. 764	0. 712
100	1. 487	1. 462	1. 474	1. 422
200	2. 356	2. 383	2. 369	2. 317



Representative standard curve for IgG ELISA

Performance Characteristics

SENSITIVITY: The minimum detectable dose was 1.5ng/mL.

SPECIFICITY: This assay recognizes both natural and recombinant rat IgG. The factors listed below were prepared at 100ng/ml in Standard /sample Diluent and assayed for cross-reactivity and no significant cross-reactivity or interference was observed.

Factors assayed for cross-reactivity

Recombinant rat	Recombinant mouse	Recombinant human
IL-2		
IL-4		
IL-6		
IL-8		

REPEATABILITY: The coefficient of variation of both intra-assay and inter-assay were less than 10%.

RECOVERY： The recovery of IgG spiked to three different levels in four samples throughout the range of the assay in various matrices was evaluated.

Recovery of IgG in two matrices

Sample Type	Average % of Expected Range (%)	Range (%)
Citrate plasma	92	83—101
Cell culture supernatants	94	85—107

LINEARITY: To assess the linearity of the assay, three samples were spiked with high concentrations of IgG in various matrices and diluted with the appropriate Sample Diluent to produce samples with values within the dynamic range of the assay. (The plasma samples were initially diluted 1:1)

Dilution ratio	Recovery (%)	Citrate plasma	Cell culture supernatants
1:2	Average% of Expected	**96**	103
	Range (%)	88—105	93—114
1:4	Average% of Expected	95	104
	Range (%)	87—104	96—113
1:8	Average% of Expected	**96**	107
	Range (%)	87—106	98—116
1:16	Average% of Expected	**98**	106
	Range (%)	89—106	98—118

REFERENCES

1. Stadlmann J, et al. (2008). Proteomics 8 (14): 2858.

2. Meulenbroek, A.J. et al. (1996).