Goat IgG ELISA Kit (Goat) (OKCA00272)

Instructions for Use

For the quantitative measurement of Goat IgG in serum, plasma.

This product is intended for research use only.

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1. Background

Principle

Aviva Systems Biology Goat IgG ELISA Kit (Goat) (OKCA00272) is based on a competitive enzyme immuno assay technique. The microtiter well-plate in this kit has been pre-coated with Goat IgG and blocked. Standards or samples are added to the plate wells along with a horseradish peroxidase (HRP) conjugated antibody specific to Goat IgG and incubated. Goat IgG immobilized in the wells competes with the liquid phase Goat IgG contained in the samples or standards for binding with the HRP-Conjugated Goat IgG Antibody. Wells are washed removing unbound Goat IgG, unbound HRP-Conjugated Goat IgG Antibody or Goat IgG /HRP-Conjugated Goat IgG Antibody complex. HRP-Conjugated Goat IgG Antibody bound to Goat IgG immobilized in the well remains. An enzymatic reaction is produced through the addition of TMB substrate which is catalyzed by the immobilized HRP to generate a blue color product that changes to yellow after adding acidic stop solution. The density of yellow coloration is measured by reading the absorbance at 450 nm which is quantitatively proportional to the amount of HRP-Conjugated Goat IgG Antibody captured in the well and inversely proportional to the amount of Goat IgG which was contained in the sample or standard.

2. Precautions

• Read instructions fully prior to beginning use of the assay kit.
• Any deviations or modifications from the described method or use of other reagents could result in a reduction of performance.
• Reduce exposure to potentially harmful substances by wearing personal protective lab equipment including lab coats, gloves and glasses.
• For information on hazardous substances included in the kit please refer to the Material Safety Data Sheet (MSDS).
• Kit cannot be used beyond the expiration date on the label.

3. Storage and Stability

• Upon receipt store kit at 4°C for 6 months or -20°C for 12 months. Avoid multiple freeze/thaw cycles.

4. Kit Components

• The following reagents are the provided contents of the kit.

Description	Quantity	Storage Conditions
Goat IgG Microplate	96 Well (12 x 8 Well Strips)	2-8°C for 1 Months
Goat IgG Standard	6 x 500 µL	2-8°C for 1 Months -20°C long term
100X Anti- Goat IgG Antibody HRP Conjugate	1 x 6 mL	
Sample Diluent	2 x 20 mL	2-8°C for 1 Months
25X Wash Buffer	1 x 20 mL	
TMB Substrate	1 x 10 mL	
Stop Solution	1 x 10 mL	

5. Required Materials Not Supplied

• Microplate reader capable of reading absorbance at 450 nm.
• Automated plate washer (optional).
• Pipettes capable of precisely dispensing 0.5 µL through 1 mL volumes of aqueous solutions.
• Pipettes or volumetric glassware capable of precisely measuring 1 mL through 100 mL of aqueous solutions.
• New, clean tubes and/or micro-centrifuge tubes for the preparation of standards or samples.
• Absorbent paper or paper toweling.
• Distilled or deionized ultrapure water.
• 37°C Incubator (optional)

6. Technical Application Tips

• Do not mix or substitute components from other kits.
• To ensure the validity of experimental operation, it is recommended that pilot experiments using standards and a small selection of sample dilutions to ensure optimal dilution range for quantitation.
• Samples exhibiting OD measurements higher than the highest standard should be diluted further in the appropriate sample dilution buffers.
• Prior to using the kit, briefly spin component tubes to collect all reagents at the bottom.
• Replicate wells are recommended for standards and samples.
• Cover microplate while incubating to prevent evaporation.
• Do not allow the microplate wells dry at any point during the assay procedure.
• Do not reuse tips or tube to prevent cross contamination.
• Avoid causing bubbles or foaming when pipetting, mixing or reconstituting.
• Completely remove of all liquids when washing to prevent cross contamination.
• Prepare reagents immediately prior to use and do not store, with the exception of the top standard.
• Equilibrate all materials to ambient room temperature prior to use (standards exception).
• For optimal results in inter- intra-assay consistency, equilibrate all materials to 37°C prior to performing assay (standards exception) and perform all incubations at 37°C.
• Pipetting less than 1 µL is not recommended for optimal assay accuracy.
• Once the procedure has been started, all steps should be completed without interruption. Ensure that all reagents, materials and devices are ready at the appropriate time.
• Incubation times will affect results. All wells should be handled in the same sequential order and time intervals for optimal results.
• Samples containing precipitates or fibrin strands or which are hemolytic of lipemic might cause inaccurate results due to interfering factors.
• TMB Substrate is easily contaminated and should be colorless or light blue until added to plate. Handle carefully and protect from light.

7. Reagent Preparation

• Equilibrate all materials to room temperature prior to use and use prepare immediately prior to use.

7.1 1X Anti-Goat IgG Antibody HRP-Conjugate

Provided at ready to use concentration.

7.2 Microplate Preparation

• Micro-plates are provided ready to use and do not require rinsing or blocking.
• Unused well strips should be returned to the original packaging, sealed and stored at °4C.
• Equilibrate microplates to ambient temperatures prior to opening to reduce potential condensation.

7.3 Goat IgG Assay Standards

Provided at ready to use concentration. Standard concentrations are as follows:

Tube	Standard 5	Standard 4	Standard 3	Standard 2	Standard 1	Standard 0
Concentration (µg/mL)	37.50	9.38	2.34	0.59	0.15	0

7.4 1X Wash Buffer

7.4.1 If crystals have formed in the **25X Wash Buffer** concentrate, equilibrate to room temperature and mix gently until crystals have completely dissolved.

7.4.2 Add the entire 20 mL contents of the **25X Wash Buffer** bottle to 480 mL of ultra-pure water to a clean > 500 mL bottle or other vessel.

7.4.3 Seal and mix gently by inversion. Avoid foaming or bubbles.

7.4.4 Store the **1X Wash Buffer** at room temperature until ready to use in the procedure. Store the prepared **1X Wash Buffer** at 4°C for no longer than 1 week. Do not freeze.

8. Sample Preparation

8.1 Sample Preparation and Storage

- Store samples to be assayed at 2-8°C for 24 hours prior being assayed.
- For long term storage, aliquot and freeze samples at -20°C. Avoid repeated freeze-thaw cycles.
- Samples not indicated in the manual must be tested to determine if the kit is valid.
- Prepare samples as follows:

    - **Serum** - Use a serum separator tube (SST) and allow samples to clot for two hours at room temperature or overnight at 4°C before centrifugation for 15 minutes at 1,000 x g. Remove serum and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.
    - **Plasma** - Collect plasma using EDTA, or heparin as an anticoagulant. Centrifuge for 15 minutes at 1,000 x g at 2-8°C within 30 minutes of collection. Assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

8.2 Sample Dilution

Target protein concentration must be estimated and appropriate sample dilution selected such that the final target protein concentration falls near the middle of the assay linear dynamic range.

- Recommended sample dilution for serum or plasma samples is 1:20,000 using **Sample Diluent**.
- The suggested 20,000-fold dilution can be prepared by serially dilution. First add 1 µL sample to 99 µL of **Sample Diluent**. Then add 2 µL of this dilution to 398 µL of **Sample Diluent**.
- Mix diluted samples gently and thoroughly.
- Pipetting less than 2 µL is not recommended for optimal assay accuracy.
- Optimal dilution must be determined by the user according to their specific samples.

9. Assay Procedure

• Equilibrate all reagents and materials to ambient room temperature prior to use in the procedure.
• To control for small potential variations in micro well-plate and day to day ambient temperature fluctuations, equilibrate all reagents prior to use and perform all incubation steps at to 37°C for optimal consistency and reproducibility.

9.1 Determine the required number of wells and return any remaining unused wells and desiccant to the pouch.

9.2 Retain at least one well as an absolute Blank without any samples or reagents.

9.3 Add 50 µL of serially titrated standards, diluted samples or blank into wells of the **Goat IgG Microplate**. At least two replicates of each standard, sample or blank is recommended.

9.4 Immediately add 50 µL of **1X Anti- Goat IgG Antibody HRP-Conjugate** to each well (excluding absolute Blank).

9.5 Cover the plate with the well plate lid and incubate for 40 minutes.

9.6 Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle or aspiration.

9.7 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time

9.8 Wash plate five times with **1X Wash Buffer** as follows:

9.8.1 Add 300 µL of **1X Wash Buffer** to each assay well.

9.8.2 Incubate for 2 minutes.

9.8.3 Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle or aspiration.

9.8.4 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.

9.8.5 Repeat steps 10.8.1 through 10.8.4 **four** more times.

9.9 Add 90 µL of **TMB Substrate** to each well and incubate **in the dark** for 20 minutes. Wells should change to gradations of blue. If the color is too deep, reduce the incubation time.

(NOTE: optimal incubation time must be determined by the user. Optimal development can be visualized by blue shading in the top four standard wells, while the remaining standards still apear clearer.)

9.10 Add 50 µL of **Stop Solution** to each well. Well color should change to gradations of yellow immediately. Add the **Stop Solution** in the same well order as done for the **TMB Substrate**.

9.11 Read the O.D. absorbance at 450 nm with a standard microplate reader within 5 minutes of stopping the reaction in step 10.10. If wavelength correction is available, set to 540 nm or 570 nm.

10. Calculation of Results

For analysis of the assay results, calculate the **Relative OD_{450}** for each test or standard well as follows:

$$(\text{Relative } OD_{450}) = (\text{Well } OD_{450}) - (\text{Mean Blank Well } OD_{450})$$

The standard curve is generated by plotting the mean replicate **Relative OD_{450}** of each standard serial dilution point vs. the respective standard concentration. The Goat IgG concentration contained in the samples can be interpolated by using linear regression of each mean sample **Relative OD_{450}** against the standard curve. This is best achieved using curve fitting software.

Note: if wavelength correction readings are available, subtract the readings at 540 nm or 570 nm from the readings at 450 nm. This may provide greater reading accuracy.

Note: if the samples measured were diluted, multiply the derived mean sample concentration by the dilution factor for a final sample concentration.

11. Typical Expected Data

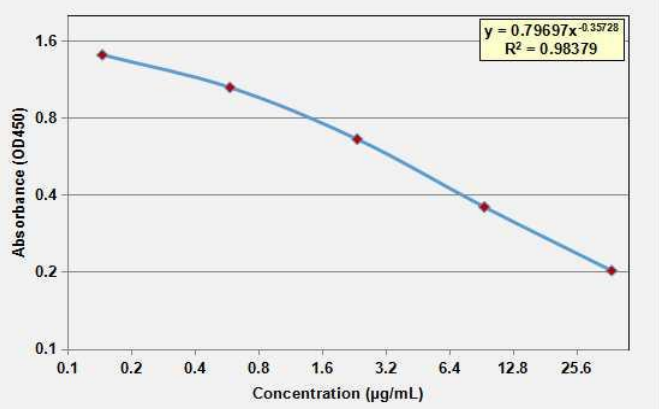
11.1 Typical absorbance values

Expected absorbance for standards when TMB incubation is performed for 20 minutes at 37°C and measured at OD_{450}.

Sample Concentration (µg/mL)	Sample 1 (OD_{450})	Sample 2 (OD_{450})	Mean (OD_{450})
37.5	0.211	0.193	0.202
9.375	0.37	0.345	0.358
2.344	0.633	0.685	0.659
0.586	1.052	1.046	1.049
0.146	1.455	1.356	1.406
0	1.721	1.714	1.718

11.2 Typical standard curve

This standard curve is for demonstration purposes only. An assay specific standard curve should be performed with each assay.



11.3 General Specifications

General Specifications	
Range	0.15µg/mL – 37.5µg/mL
LOD	< 0.134 µg/mL (Derived by linear regression of OD_{450} of the Mean Blank + 2xSD)
Specificity	Goat IgG
Cross-Reactivity	No significant cross-reactivity or interference between goat IgG and analogues was observed.

11.4 Reproducibility

Three samples concentrations were measured in replicate within an assay plate and across replicate assays to assess Intra- and Inter-Assay precision.

Intra-Assay Precision: %CV < 8%, n=20

Inter-Assay Precision: %CV < 10%, n=20

11.5 Linearity

Sample matrices (indicated below) were spiked with known concentrations of Goat IgG, diluted to within the dynamic range of the assay and measured to assess the linearity of the assay measurements across the range of dilution points.

Sample Type	Sample Dilution	Average Recovery	Range
Serum (n=4)	1:2,000	85%	80-94%
	1:4,000	88%	83-99%
	1:8,000	92%	86-104%
	1:16,000	96%	89-108%

12. Technical Resources

Technical Support:

For optimal service please be prepared to supply the lot number of the kit used.

USA

Aviva Systems Biology, Corp.
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