



Total Goat IgG

Immunoenzymetric Assay for the Measurement of Total Goat Immunoglobulin G Catalog # F210

Intended Use

This kit is intended for use in quantifying very low concentrations of total goat immunoglobulin G (goat IgG). The kit is for Research and Manufacturing Use Only and is not intended for diagnostic use in humans or animals.

Summary and Explanation

The sensitive and specific detection and quantitation of very low levels of goat IgG as low as a few picograms/mL can be accomplished by the use of a two-site immunoenzymetric assay, also termed ELISA. The specificity of the antibodies used in this kit for goat IgG, heavy and light chain, means that the assay will provide accurate results for goat IgG even in the presence of other animal proteins. As such this assay is useful in detecting trace contamination by goat IgG in a variety of sample types including transgenic products.

Principle of the Procedure

The goat IgG assay is a two-site immunoenzymetric assay. Samples which may contain goat IgG are reacted in microtiter strips coated with an affinity purified capture antibody. A second, horse radish peroxidase (HRP) enzyme labeled anti-goat IgG antibody, is reacted simultaneously resulting in the formation of a sandwich complex of solid phase antibody-goat IgG-enzyme labeled antibody. The microtiter strips are washed to remove any unbound reactants. The substrate, tetramethyl benzidine (TMB) is then reacted. The amount of hydrolyzed substrate is read on a microtiter plate reader and is directly proportional to the concentration of goat IgG present.

Storage & Stability

- * All reagents should be stored at 2°C to 8°C for stability until the expiration date printed.
- * The substrate reagent should not be used if its stopped absorbance at 450nm is greater than 0.1.
- * Reconstituted wash solution is stable until the expiration date of the kit.

Reagents & Materials Provided

<u>Component</u>	<u>Product #</u>
Anti-goat IgG:HRP Affinity purified goat antibody to goat IgG, H&L chains, conjugated to HRP in a protein matrix with preservative, 1x12mL	F211
Anti-goat IgG coated microtiter strips 12x8 well strips in a bag with desiccant	F212
Goat IgG Standards Highly purified goat IgG in a protein matrix with preservative. Standards at 0, 0.5, 1.5, 5, 15, & 50ng/mL, 1mL/vial	F213
TMB Substrate 3,3',5,5' Tetramethyl benzidine, 1x12mL	F005
Wash Concentrate (20X) Tris buffered saline with preservative, 1x50mL	F004
Stop Solution 0.5N sulfuric acid, 1x12mL	F006

Materials & Equipment Required But Not Provided

Microtiter plate reader spectrophotometer with dual wavelength capability at 450 & 650nm
(If your plate reader does not provide dual wavelength analysis you may read at just the 450nm wavelength.)
Microtiter plate washing system
Pipettors - 50µL and 100µL
Repeating or multichannel pipettor - 100µL
Microtiter plate rotator (150 - 200 rpm)
Distilled water
1 liter container for wash solution storage

Precautions

- For research or manufacturing use only.
- Stop reagent is 0.5N H₂SO₄. Avoid contact with eyes, skin, and clothing. At the concentrations used in this kit none of the other chemical reagents are believed to be harmful.
- This kit should only be used by qualified technicians.

Preparation of Reagents

- * Bring all reagents to room temperature.
- * Dilute wash concentrate to 1 liter in distilled water, label with kit lot/expiration date, and store at 4°C.

Procedural Notes

1. Complete washing of the plates to remove excess unreacted reagents is essential to good assay reproducibility and sensitivity. We advise against the use of automated or other manual operated vacuum aspiration devices for washing plates as these may result in lower specific absorbances, higher non-specific absorbance, and more variable precision. The manual wash procedure described below generally provides lower backgrounds, higher specific absorbance, and better precision. If duplicate CVs are poor, or if the absorbance of the 0 standard minus a substrate blank is greater than 0.200, evaluate plate washing procedure for proper performance.
2. Dilution of samples will be required for samples >50ng/mL. Proposed diluents should be validated in the assay to demonstrate that they do not give elevated background and are not contaminated with goat IgG. The diluent should also give acceptable recovery when spiked with known quantities of goat IgG. Alternatively, *Cygnus Technologies* sells a diluent validated for this assay, Catalog # F213A.
3. High Dose Hook Effect may be observed in samples with very high concentrations of goat IgG. Samples greater than 10,000ng/mL may give absorbances less than the 50ng/mL standard. High Dose Hook samples are most likely to be encountered in samples derived from human serum or blood. If a hook effect is possible samples should also be assayed diluted. If the absorbance of the undiluted sample is less than the diluted samples this may be indicative of the hook effect. Such samples should be diluted until the dilution adjusted value remains constant.
4. Because this kit is an extremely sensitive assay for goat IgG, it is possible to inadvertently contaminate the kit reagents with various external sources of goat IgG. Such goat IgG contamination can arise from use of pipetting or other laboratory equipment or surfaces which have come into contact with more concentrated forms of goat products. Airborne contamination from concentrated sources will also easily contaminate the kit reagents and potentially give false values and or poor assay reproducibility. Take precautions to minimize contamination.

Limitations

- * Certain sample matrices may interfere in this assay. Although the assay is designed to minimize matrix

interference, materials such as detergents in high concentration or extremes of pH (<5.0 and >8.5) or very high drug product protein concentrations may give erroneous results. It is advised to test the sample matrix for interference by diluting the 50ng/mL standard 1 part to 4 parts of the matrix containing no goat IgG contaminants. This diluted standard when assayed as an unknown should give a value of 8 to 12ng/mL. Consult *Cygnus Technologies* Technical Service Department for advice on how to quantitate the assay in problematic matrices. If sensitivity requirements allow, one solution may be to dilute all samples in an approved diluent prior to assay. Alternatively it may prove necessary to make standards or calibrators in the same matrix as the sample.

- * Avoid the assay of samples containing sodium azide (NaN₃) which will destroy the HRP activity of the conjugate and could result in the under-estimation of goat IgG levels.

Assay Protocol

- * The protocol recommended below was followed to generate the data found in the **Performance Characteristics** section. The assay protocol may be modified to achieve other operator desired characteristics. For example, sensitivity can be significantly improved by increasing sample size and/or by increasing the immunological incubation time. Consult *Cygnus Technologies* Technical Service Department for advice on how to modify the protocol to best suite your particular performance goals.
- * The protocol specifies the use of an approved microtiter plate shaker or rotator for the immunological step. If you do not have such a device it is possible to incubate the plate without shaking however it will be necessary to extend the first immunological incubation step by about 30 minutes to achieve comparable results to the 2 hour shaking protocol. **Do not shake during the 30 minute substrate incubation step as this may result in higher backgrounds and worse precision.** Bring all reagents to room temperature. Set-up plate spectrophotometer to read dual wavelength at 450nm for the test wavelength and 650nm for the reference.
- * Thorough washing is essential to proper performance of this assay.
- * All standards, controls, and samples should be assayed in duplicate.
- * Maintain a repetitive timing sequence from well to well for all assay steps to insure that all incubation times are the same for each well.
- * Make a work list for each assay to identify the location of each standard, control, and sample.

* If the substrate has a distinct blue color prior to the assay it may have been contaminated. If the absorbance of 100µL of substrate plus 100µL of stop against a water blank is greater than 0.1 it may be necessary to obtain new substrate or the sensitivity of the assay may be compromised.

* Strips should be read within 30 minutes after adding stop solution since color will fade over time.

Assay Protocol

1. Pipette 50µL of standards, controls and samples into wells indicated on work list.

2. Pipette 100µL of anti-goat IgG:HRP (#F211) into each well.

3. Cover or place in a zip-lock plastic bag, transfer to rotator, and incubate for 2 hours at 180rpm, at room temperature, 24°C ± 4°.

4. Dump contents of wells into waste or gently aspirate with a pipettor. Blot and vigorously bang out residual liquid over absorbent paper. Fill wells generously with diluted wash solution by flooding well from a squirt bottle or by pipeting in ~350 µL. Dump and bang again. Repeat for a total of 4 washes. Wipe off any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. Do not allow wash solution to remain in wells for longer than a few seconds. Do not allow wells to dry before adding TMB substrate.

5. Pipette 100µL of substrate.

6. Incubate for 30 minutes. Do Not Shake.

7. Pipette 100µL of stop solution.

8. Read absorbance at 450/650nm blanking on the Zero standard.

Calculation of Results

The standards may be used to construct a standard curve with values reported in ng/mL. This data reduction may be performed through computer

methods using curve fitting routines such as point to point, spline, or polynomial methods. Linear regression fitting is not recommended as the method from which to interpolate unknowns. Data may also be manually reduced by plotting the absorbance values of the standard on the y-axis versus concentration on the x-axis and drawing a smooth point to point line. Absorbances of samples are then interpolated from this standard curve. Be sure to correct all diluted sample values by multiplying by the dilution factor.

Quality Control

- Precision on duplicate samples should yield average % coefficients of variation of less than 10% for samples greater than 0.5 ng/mL and < 50 ng/mL. CVs for samples < 0.5 ng/mL may be greater than 10%.

- For optimal performance the absorbance of the substrate when blanked against water should be <0.1.

- It is recommended that each laboratory assay appropriate quality control samples in each run to insure that all reagents and procedures are correct.

Example Data

Well #	Contents	Abs. at 450nm	Mean Abs.	ng/mL goat IgG
1A	Zero Std	0.000		
1B	Zero Std	0.002	0.001	
1C	0.5ng/mL	0.020		
1D	0.5ng/mL	0.024	0.022	
1E	1.5ng/mL	0.071		
1F	1.5ng/mL	0.065	0.068	
1G	5 ng/mL	0.218		
1H	5 ng/mL	0.238	0.228	
2A	15 ng/mL	0.680		
2B	15 ng/mL	0.640	0.660	
2C	50 ng/mL	2.144		
2D	50 ng/mL	2.052	2.098	
2E	sample A	2.255		
2F	sample A	2.215	2.235	>50
2G	sample B	0.072		
2H	sample B	0.070	0.071	1.7

Performance Characteristics

Cygnus Technologies has validated this assay by conventional criteria as indicated below. This validation is generic in nature and is intended to supplement but not replace certain user and product or sample specific validation which should be performed by each laboratory.

Sensitivity

The lower limit of detection (**LOD**) is defined as that concentration corresponding to a signal two standard deviations above the mean of the zero standard. LOD is <0.2 ng/mL.

The lower limit of quantitation (**LOQ**) is defined as the lowest concentration where concentration coefficients of variation (CVs) are <20%.

LOQ is ~0.3 ng/mL.

Precision

Both intra (n=20 replicates) and inter-assay (n=5 assays) precision were determined on 3 pools with low (1.50ng/mL), medium (~5.08ng/mL), and high concentrations (~13.05ng/mL). The % CV is the standard deviation divided by the mean and multiplied by 100.

Pool	Intra assay CV	Inter assay CV
Low	4.7%	6.2%
Medium	6.0%	5.7%
High	5.4%	5.7%

Specificity/Cross-Reactivity

Cross reactivity in two site ELISAs can manifest itself as either a false increase in goat IgG levels (positive cross reactivity) or as a false decrease in true goat IgG (negative cross reactivity). Animal IgG fractions and serum from the various animal species shown below were tested for positive cross reactivity by assaying the sample as an unknown. Negative cross reactivity was evaluated by spiking 25 ng/mL of goat IgG into each of the potential cross reactants and dividing the recovered value by 25 ng/mL. Of the materials below only bovine and sheep IgG should show some positive cross reactivity. The antibodies used in this kit have been affinity purified to minimize cross reactivity but it is recommended that each user test their particular sample matrix material for cross reactivity in a similar experiment.

Animal Species	% Cross Reactivity
Cat	Not detectable
Chicken	Not detectable
Cow	40%
Dog	Not detectable
Duck	Not detectable
Guinea pig	Not detectable
Hamster, Syrian	Not detectable
Horse	Not detectable
Human	Not detectable
Mouse	Not detectable
Pig	Not detectable
Rabbit	Not detectable
Rat	Not detectable
Sheep	100%

Because the antibodies used in this kit were generated and affinity purified against goat IgG heavy and light chains, there is some cross reactivity to other goat

immunoglobulin classes such as goat IgA, and goat IgM.

Recovery/ Interference Studies

Various buffer matrices have been evaluated by adding known amounts of the goat IgG preparation used to make the standards in this kit. Because this assay is designed to minimize matrix interference most of these buffers yielded acceptable recovery defined as between 80-120%. In general, extremes in pH (<5.0 and >8.5) as well as some detergents like SDS and Tween can cause under-recovery. Very high concentrations of certain proteins can also interfere in accurate detection of goat IgG. Each user should validate that their sample matrices yield accurate recovery by performing a similar experiment. For example, this experiment can be performed by diluting one part of the 50ng/mL standard provided with this kit into 4 parts of the sample matrix in question. Recovery should be on the order of 8 to 12 ng/mL goat IgG. Consult *Cygnus Technologies* Technical Services if you have recovery problems in your matrix.

Hook Capacity

Increasing concentrations of goat IgG >50 ng/mL were assayed as unknowns. The hook capacity, defined as that concentration which will give an absorbance reading less than the 50 ng/mL standard was ~30 µg/mL.

Ordering Information/ Customer Service

To place an order or to obtain additional product information contact *Cygnus Technologies* Customer Support:

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