Cygnus Technologies, Inc.
Revision #8-05

Total Mouse IgG Immunoglobulin Assay

Immunoenzymetric Assay for the Measurement of
Total Mouse IgG Immunoglobulin
Catalog # F049

Intended Use

This kit is intended for use in quantitating total mouse IgG. The antibodies used in this kit are specific for mouse immunoglobulins and will not cross react significantly with most immunoglobulins from other species. The kit is for Research and Manufacturing use only and is not intended for diagnostic use in humans or animals.

Summary and Explanation Of the Test

Monoclonal antibodies are used in many applications such as diagnostic or therapeutic agents as well as affinity supports for immunoaffinity purification of various products. These applications frequently require a sensitive assay to accurately quantitate the concentration of these antibodies in complex sample matrices. The antibodies used in this kit are mouse immunoglobulin heavy and light chain specific. They do not cross react significantly with immunoglobulin from other species but they will cross react to varying degrees with other mouse immunoglobulins such as all IgG isotypes, IgA, and IgM. The presence of high concentrations of mouse IgM and IgA immunoglobulin types in the sample can interfere in the ability of this kit to quantitate mouse IgG. ***Cygnus Technologies*** provides kits for IgA, IgG1, IgG2a, IgG2b, IgG3 and IgM immunoglobulins. See ordering information on the last page.

Principle of the Procedure

The Mouse IgG assay is a two-site immunoenzymetric assay. Samples containing mouse IgG are reacted in microtiter strips coated with an affinity purified capture antibody. A second alkaline phosphatase enzyme labeled goat anti-mouse immmunoglobulin antibody is reacted, forming a sandwich complex of solid phase antibody-mouse immunoglobulin-enzyme labeled antibody. The microtiter strips are then washed to remove any unbound reactants. The substrate p-nitrophenyl phosphate (PNPP) is then reacted. The amount of hydrolyzed substrate is read on a microtiter plate reader and will be directly proportional to the concentration of mouse IgG present. Accurate quantitation is achieved by comparing the signal of unknowns to IgG standards assayed at the same time.

Reagents & Materials Provided

Component	Product #
Anti-mouse immunoglobulin:	
Alkaline Phosphatase Affinity purified goat antibody in a protein matrix with preservative, 1x22mL	**F076**
Anti-mouse coated microtiter strips 12x8 well strips in a bag with desiccant	**F075**
Mouse IgG Standards Mouse IgG in a bovine protein matrix with preservative. Standards at 0, 0.25, 1, 4, and 20 ng/mL, 1mL/vial	**F081**
PNPP Substrate p-nitrophenyl phosphate in a Diethanolamine buffer with preservative, 1x22mL	**F008**
Wash Concentrate (20X) Tris buffered saline with preservative, 1x50mL	**F004**

Storage & Stability

* All reagents should be stored a 2°C to 8°C for stability until the expiration date printed.
* The substrate reagent should not be used if its absorbance at 405nm is greater than 0.4.
* Reconstituted wash solution is stable until the expiration date of the kit.

Materials & Equipment Required But Not Provided

Microtiter plate reader spectrophotometer with dual wavelength capability at 405 & 492nm.
(If your plate reader does not provide dual wavelength analysis you may read at just the 405nm wavelength.)
Pipettors - 50µL and 100µL
Repeating or multichannel pipettor - 100µL
Microtiter plate rotator (150 - 200 rpm)
Sample Diluent (Recommend Cygnus Cat # I028)
Distilled water
1 liter wash bottle for diluted wash solution

Precautions

For research or manufacturing use only. At the concentrations used in this kit none of the 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, and label with kit lot and 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 0.15, evaluate plate washing procedure for proper performance.
2. High Dose Hook Effect may be observed in samples with very high concentrations of mouse immunoglobulin. Samples greater than 20µg/mL may give absorbances less than the 20ng/mL standard. Hook effect is indicated when absorbance of the undiluted sample is less than the diluted samples. If hook effect is possible, samples should also be assayed diluted.
3. When dilution of samples is required dilution should be performed in a diluent validated to yield acceptable background and not contaminated with mIgG. The diluent should also give acceptable recovery when spiked with known quantities of mIgG. The preferred diluent is our Cat# I-028 available in 100mL, 500mL, or 1 liter bottles. This is the same material used to prepare the kit standards. As the sample is diluted in I-028 its matrix begins to approach that of the standards thus reducing any inaccuracies caused by dilutional artifacts. Other prospective diluents must be tested for recovery by using them to dilute the 20ng/mL standard, as described in the "Limitations" section below.
4. If the substrate has a distinct yellow color prior to performing the assay it may have been contaminated. If this appears to be the case read 200µL of substrate against a water blank. If the absorbance is greater than 0.4 it may be necessary to obtain new substrate or the sensitivity of the assay may be compromised. The PNPP substrate is very sensitive to environmental contamination. Do not leave bottle open or at room temperature for longer than is needed. Only remove as much reagent as is needed for your assay run and do not return any unused substrate back into the substrate bottle. Additional substrate can be purchased separately as Cat # F008.

Limitations

* The antibodies used in this kit crossreact with mouse IgM & IgA at approximately 10% on a molar basis.
* Certain sample matrices may interfere in this assay. Although the assay is designed to minimize matrix interference, materials such as detergents in high concentration, extremes of pH (<6.0 and >8.5), very high buffer molarity, or very high protein concentrations may give erroneous results. **For these reasons we recommend that you first establish acceptable recovery in your sample matrices by performing a dilution/recovery experiment.**
This test can be very simply performed by diluting 1 part of the 20ng/mL standard supplied with the kit into 4 parts of your sample matrix that does not contain any or very low levels of mIgG. This diluted standard when assayed as an unknown should give a recovery value after correcting for any endogenous mIgG of 3 to 5 ng/mL. Consult ***Cygnus Technologies*** Technical Service Department for advice on how to quantitate the assay in problematic matrices.

Assay Protocol

*The assay is very robust such that assay variables like incubation times, sample size, and other sequential incubation schemes can be altered to manipulate assay performance for more sensitivity, increased upper analytical range, or reduced sample matrix interference. Increasing incubation time for the PNPP substrate step will in general increase absorbances proportionately for all wells. For example, doubling the substrate step time from 60 minutes to 120 minutes will double all ODs. Before modifying the protocol from what is recommended, users are advised to contact our technical services for input on the best way to achieve your desired 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 60 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 405nm for the test wavelength and 492nm for the reference wavelength. Blank the instrument using the zero standard wells after assay completion.
* 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 yellow color prior to the assay it may have been contaminated. If this appears to be the case read 200µL of substrate against a water blank. If the absorbance is greater than 0.4 it may be necessary to obtain new substrate or the sensitivity of the assay may be compromised.
*Samples containing mIgG greater than 20ng/mL should be diluted in an appropriate diluent. (See Procedural Note # 2) Be sure to multiply diluted sample concentrations by the dilution factor when calculating the results.

Protocol

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

2. Pipette 200µL of anti-mouse immunoglobulin: alkaline phosphatase into each well.

3. Cover & incubate on rotator at ~ 180rpm for 2 hours at room temperature.

4. Dump the contents into waste or gently aspirate using a multi-channel pipetor. Blot and bang out residual liquid over low lint absorbance paper. Wash generously with diluted wash solution by flooding the wells with solution from a squirt bottle or by pipetting in ~350µL. Repeat for a total of 4 times. Wipe off any liquid from the bottom outside of the wells as any residue can interfere in the reading step.

5. Pipette 200µL of substrate.

6. Incubate for 60 minutes. DO NOT SHAKE!

7. Read absorbance at 405/492nm blanking on the Zero standard.

Calculation of Results

The standards may be used to construct a standard curve with values reported in ng/mL. (See Limitations section above). This data reduction may be performed through computer methods using curve fitting routines such as point to point, spline, or 4 parameter logistic fit. **Do not use linear regression analysis to interpolate values for samples as this may lead to significant inaccuracies!** 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.

Example Data

Well #	Contents	Abs. at 405nm	Mean Abs.	ng/mL IgG
1A	Zero Std	0.000		
1B	Zero Std	0.002	0.001	
1C	0.25ng/mL	0.027		
1D	0.25ng/mL	0.026	0.027	
1E	1ng/mL	0.103		
1F	1ng/mL	0.100	0.102	
1G	4ng/mL	0.485		
1H	4ng/mL	0.495	0.490	
2A	20ng/mL	1.757		
2B	20ng/mL	1.739	1.748	
2C	sample 1	0.005		
2D	sample 1	0.010	0.008	<0.2ng
2E	sample 2	0.100		
2F	sample 2	0.105	0.103	1ng

Quality Control

-Precision on duplicate samples should yield average % coefficients of variation of less than 10% for samples greater than 1ng/mL. CVs for samples < 1ng/mL may be greater than 10%.
- For optimal performance the absorbance of the substrate when blanked against water should be < 0.4.
- It is recommended that each laboratory assay appropriate quality control samples in each run to insure that all reagents and procedures are correct.

Ordering Information/ Customer Service

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

Tel: 910-454-9442 Fax: 910-454-9443
Email: cygnustec@aol.com
Website: www.cygnustechnologies.com
4705 Southport Supply Road, Suite 208
Southport, NC 28461 USA

For other mouse immunoglobulin kits please specify the following catalog numbers:

IgG1	Cat. # F045
IgG2a	Cat. # F046
IgG2b	Cat. # F047
IgG3	Cat. # F200
IgM	Cat. # F090