Product Manual

IgG_1 (mouse), ELISA kit

Catalog #: *ADI-900-109A*

96 well Enzyme-Linked Immunosorbent Assay Kit

For use with serum and culture supernatants

For the latest product information, including support documentation, visit us online:
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Product Manual

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Carefully note the handling and storage conditions of each kit component.

INTRODUCTION

The IgG_1 (mouse), ELISA kit is a complete kit for the quantitative determination of mouse IgG_1 in culture supernatants and serum. Please read the complete kit insert before performing this assay. The kit uses a polyclonal antibody to mouse IgG immobilized on a microtiter plate to bind the mouse IgG in the standards or sample. A mouse IgG_1 Standard is provided in the kit. After a simultaneous incubation with a polyclonal antibody to mouse IgG_1 conjugated to Horseradish peroxidase, which binds to the mouse IgG_1 captured on the plate, the excess reagents are washed out and substrate is added. After a short incubation, the enzyme reaction is stopped and the color generated is read at 450 nm. The measured optical density is directly proportional to the concentration of mouse IgG_1 in either standards or samples. For further explanation of the principles and practices of immunoassays please see the excellent books by Chard[1] or Tijssen[2].

IgG is divided into four subclasses; IgG_1, IgG_2, IgG_3, and IgG_4. IgG_1 is the most abundant immunoglobulin found in the blood. It is a glycoprotein which consists of two identical heavy chains (50 kDal each) and two identical light chains (25 kDal each), to give a combined mass of approximately 150 kDal. The chains are held in place by covalent disulfide bonds. Each light chain contains two immunoglobulin (Ig) domains, while the heavy chains contain four Ig domains each. In the middle of each heavy chain is a relative varying portion called the “hinge region” which is unique to each IgG. This region allows for molecular flexibility and sets IgG_1 apart from its IgG counterparts. IgG_1 properties and functions include neutralization, opsonization, activation of the complement system, diffusion into extravascular sites and crossing the placenta[3].

MATERIALS SUPPLIED

1. **Mouse IgG_1 Microtiter Plate, One Plate of 96 Wells, Component number 80-2996**
   A plate using break-apart strips coated with goat antibody specific to mouse IgG.

2. **Assay Buffer 13 Concentrate, 50 mL, Component number 80-1604**
   Tris buffered saline containing proteins and detergents.

3. **Mouse IgG_1 Conjugate Concentrate, 0.07 mL, Component number 80-2998**
   A 100X concentrate of goat anti-mouse IgG_1 conjugated to Horseradish peroxidase.

4. **Mouse IgG_1 Conjugate Diluent, 6 mL, Component number 80-2999**
   A blue buffer for dilution of mouse IgG_1 conjugate concentrate.

5. **Wash Buffer Concentrate, 100 mL, Component number 80-1287**
   Tris buffered saline containing detergents.

6. **Mouse IgG_1 Standard, 0.25 mL, Component number 80-1000**
   A solution of 5000 ng/mL mouse IgG_1.

7. **TMB Substrate, 10 mL, Component number 80-0350**
   A solution of 3,3',5,5' tetramethylbenzidine (TMB) and hydrogen peroxide. Ready to use. Protect from prolonged exposure to light.

8. **Stop Solution 2, 10 mL, Component number 80-0377**
   A 1N solution of hydrochloric acid in water. Keep tightly capped. Caution: Caustic.

9. **IgG_1 (mouse), ELISA Assay Layout Sheet, 1 each, Component number 30-0357**

10. **Plate Sealer, 2 each, Component number 30-0012**

We test this kit's performance with a variety of samples, however, it is possible that high levels of interfering substances may cause variation in assay results

The activity of the Horseradish peroxidase conjugate is affected by nucleophiles such as azide, cyanide and hydroxylamine.

The mouse IgG_1 Standard provided, Component nunber 80-1000, should be handled with care because of the known and unknown effects of IgG.

Stop Solution 2 is a 1 normal (1N) hydro-chloric acid solution. This solution is caustic; care should be taken in use.

Storage temp

STORAGE

All components of this kit, **except the conjugate concentrate**, are stable at 4°C until the kit's expiration date. The conjugate concentrate must be stored at -20°C upon receipt.

ADDITIONAL MATERIALS NEEDED

- Deionized or distilled water.
- Precision pipets for volumes between 50 µL and 1000 µL.
- Disposable test tubes for dilution of samples and standards.
- Repeater pipets for dispensing 50 µL and 100 µL.
- Disposable beakers for diluting buffer concentrates.
- Graduated cylinders.
- Plate shaker.
- Adsorbent paper for blotting.
- Microplate reader capable of reading at 450 nm, preferably with correction between 570 nm and 590 nm.
- Graph paper or software for plotting the standard curve.

SAFETY WARNINGS & PRECAUTIONS

Important/ Warning

1. Wear appropriate personnel protective apparel. Avoid contact with clothes and exposed skin. In case of accidental skin exposure, flush with water immediately. Consult a physician if required.
2. Use a safety pipetting device for all pipetting. Never pipet by mouth.
3. Interpretation of the results is the sole responsibility of the user.

SAMPLE HANDLING

If buffers other than those provided are used in the assay, the end-user must determine the appropriate dilution and assay validation.

The IgG_1 (mouse), ELISA is compatible with mouse IgG_1 culture supernatants and serum. Samples diluted sufficiently into the proper diluent can be read directly from a standard curve. Please refer to the Sample Recovery recommendations for details of suggested dilutions. Culture supernatants and serum are suitable for use in the assay. Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic specimens. Samples in the majority of culture media, including fetal bovine serum, can also be read in the assay provided the standards have been diluted into the culture media instead of Assay Buffer 13. There will be a small change in binding associated with running the standards and samples in media. Users should only use standard curves generated in media or buffer to calculate concentrations of mouse IgG_1 in the appropriate matrix. Samples must be stored frozen to avoid loss of bioactive mouse IgG_1. If samples are to be run within 24 hours, they may be stored at 4°C. Otherwise, samples must be stored frozen at -70°C to avoid loss of bioactive mouse IgG_1. Excessive freeze/thaw cycles should be avoided. Prior to assay, frozen sera should be brought to room temperature slowly and gently mixed by hand. Do not thaw samples in a 37 °C incubator. Do not vortex or sharply agitate samples.

High Dose Hook

Due to the binding nature of IgGs, a high dose hook effect will ultimately present at higher IgG concentrations. For the IgG_1 (mouse) ELISA kit, ADI-900-109A, the high dose hook effect will become noticeable at standard and sample concentrations above 250 ng/mL. Samples with concentrations above 250 ng/mL after recommended dilution may be outside the linear range of the assay. Therefore, samples diluted to the minimal recommended dilution will need to read within the standard curve range for accurate results.

PROCEDURAL NOTES

1. Do not mix components from different kit lots or use reagents beyond the kit expiration date.
2. Allow all reagents to warm to room temperature for at least 30 minutes before opening.
3. Standards can be made up in either glass or plastic tubes.
4. Pre-rinse the pipet tip with reagent, use fresh pipet tips for each sample, standard and reagent.
5. Pipet standards and samples to the bottom of the wells.
6. Add the reagents to the side of the well to avoid contamination.
7. This kit uses break-apart microtiter strips, which allow the user to measure as many samples as desired. Unused wells must be kept desiccated at 4°C in the sealed bag provided. The wells should be used in the frame provided.
8. Prior to addition of substrate, ensure that there is no residual wash buffer in the wells. Any remaining wash buffer may cause variation in assay results.
9. It is important that the matrix for the standards and samples be as similar as possible. Mouse IgG_1 samples diluted with Assay Buffer 13 should be run with a standard curve diluted in the same buffer. Serum samples should be evaluated against a standard curve run in Assay Buffer 13 while culture supernatant samples should be read against a standard curve diluted in the same complete but non-conditioned media. See Reagent Preparation, step #2.

SAMPLE RECOVERIES

Mouse IgG_1 concentrations were measured in mouse serum and tissue culture media. Mouse IgG_1 was spiked into the undiluted samples of these matrices which were then diluted with the appropriate diluent and assayed in the kit. The following results were obtained:

Sample	% Recovery*	Recommended Dilution*
Mouse Serum	102.8	1:20,000
Tissue culture media	105.3	None

REAGENT PREPARATION

1. **Wash Buffer**
   Prepare the Wash Buffer by diluting 50mL of the supplied concentrate with 950mL of deionized water. This can be stored at room temperature until the kit expiration, or for 3 months, whichever is earlier.

2. **Assay Buffer 13**
   Prepare the Assay Buffer 13 by diluting 50 mL of the supplied concentrate with 450 mL of deionized water. This can be stored at 4°C until the kit expiration, or for 3 months, whichever is earlier.

3. **Mouse IgG_1 Conjugate**
   Prepare conjugate by diluting the Conjugate Concentrate 100-fold into Conjugate Diluent. For example, dilute 60 µL of the 100x Conjugate Concentrate with 5.94 mL of Conjugate Diluent. Prepare only what is needed for each day's experiment, and discard any remaining diluted conjugate.

4. **Mouse IgG_1 Standards**
   Label six 12x75mm glass or plastic tubes #1 through #6. Pipet 475 µL of standard diluent (Assay Buffer 13 or culture media) into tube #1. Pipet 250 µL of standard diluent into tubes #2 though #6. Add 25 µL of the 5,000 ng/mL Standard to tube #1. Vortex thoroughly. Add 250 µL of tube #1 to tube #2 and vortex thoroughly. Add 250 µL of tube #2 to #3 and vortex thoroughly. Continue this for tubes #4 through #6.

   Diluted standards should be used within 60 minutes of preparation. Discard any unused standard dilutions.

   The concentration of mouse IgG_1 in tubes #1 through #6 will be 250, 125, 62.5, 31.25, 15.62, and 7.81 ng/mL respectively. See mouse IgG1 Assay Layout Sheet for dilution details.

ASSAY PROCEDURE

Bring all reagents to room temperature for at least 30 minutes prior to opening.

Refer to the Assay Layout Sheet to determine the number of wells to be used. Remove the wells not needed for the assay and return them, with the desiccant, to the mylar bag and seal. Store unused wells at 4°C.

All standards, controls and samples should be run in duplicate.

Plates will require shaking or an orbital rotor at 500 rpm.

1. Pipet 50 µL of standard diluent (Assay Buffer 13 or culture media) into the S0 (0 pg/mL standard) wells.
2. Pipet 50 µL of Standards #1 through #6 into the appropriate wells.
3. Pipet 50 µL of the Samples into the appropriate wells.
4. Add 50 µL of the diluted Conjugate to each well, except the Blank.
5. Tap the plate gently to mix the contents, and seal with the plate sealer.
6. Incubate at room temperature on a plate shaker for 1 hour.
7. Empty the contents of the wells and wash by adding ~400 µL of wash solution to every well. Repeat the wash 3 more times for a total of 4 washes. After the final wash, empty or aspirate the wells and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.
8. Pipet 100 µL of TMB Substrate into each well.
9. Incubate for 30 minutes at room temperature on a plate shaker.
10. Pipet 100 µL Stop Solution 2 to each well. This stops the reaction and the plates should be read immediately.

CALCULATION OF RESULTS

Make sure to multiply sample concentrations by the dilution factor used during sample preparation.

Several options are available for the calculation of the concentration of mouse IgG_1 in the samples. We recommend that the data be handled by an immunoassay software package (e.g. Assay Blaster! Data Analysis Software, ADI-28-0002) utilizing a 4 parameter logistic curve fitting program. If data reduction software is not readily available, the concentration of mouse IgG_1 can be calculated as follows:

1. Calculate the average net Optical Density (OD) bound for each standard and sample by subtracting the average Blank OD from the average OD for each standard and sample.

   Average Net OD = Average OD - Average Blank OD

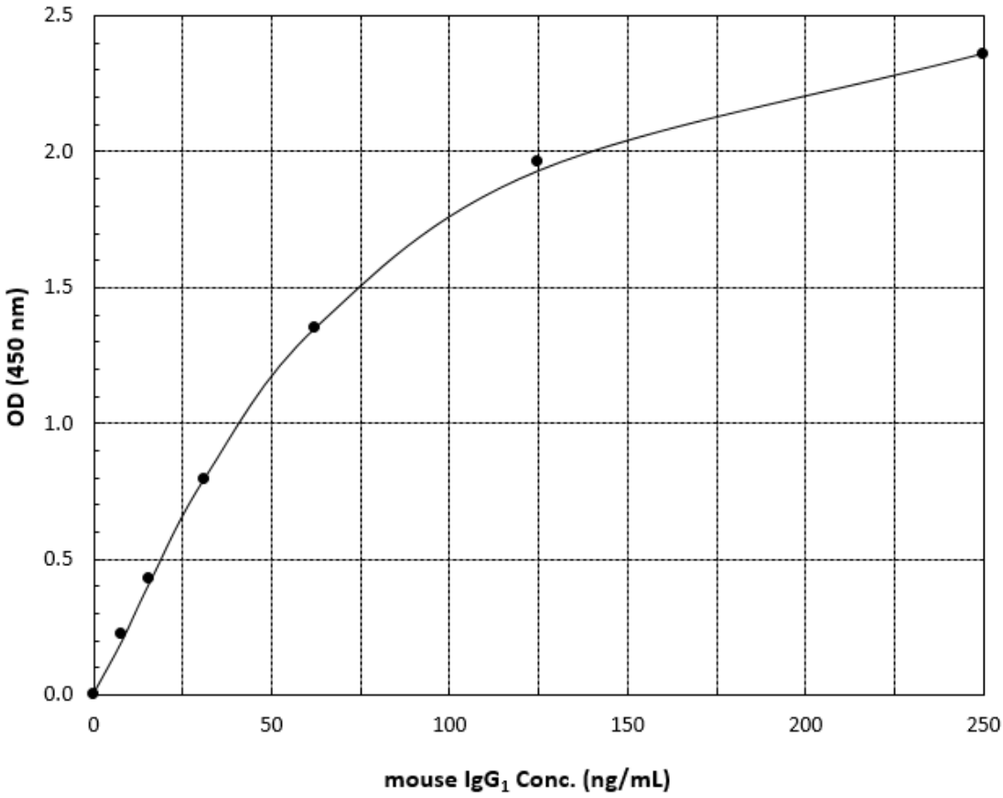
2. Using linear graph paper, plot the Average Net OD for each standard versus mouse IgG_1 concentration in each standard. Approximate a straight line through the points. The concentration of mouse IgG_1 in the unknowns can be determined by interpolation.

TYPICAL RESULTS

The results shown below are for illustration only and should not be used to calculate results from another assay.

Sample	Average Net OD	Mouse IgG_1 Conc. (ng/mL)
Blank (Mean)	0.042	
S0	0.001	**0**
S1	2.354	**250**
S2	1.964	**125**
S3	1.349	**62.5**
S4	0.793	**31.25**
S5	0.429	**15.62**
S6	0.222	**7.81**
Unknown 1	2.044	**148.7**
Unknown 2	1.731	**98.3**
Unknown 3	1.136	**48.5**

TYPICAL STANDARD CURVE



PERFORMANCE CHARACTERISTICS

The following parameters for this kit were determined using the guidelines listed in the National Committee for Clinical Laboratory Standards (NCCLS) Evaluation Protocols[4].

Sensitivity

The sensitivity or limit of detection of the assay is 0.116ng/mL and was determined by interpolation at 2 standard deviations above the background (0pg/mL) of 30 zero standard replicates

Dilutional Linearity

A sample containing 100 ng/mL mouse IgG_1 was serially diluted 4 times 1:2 in the Assay Buffer 13 supplied in the kit and measured in the assay. The data was plotted graphically as actual mouse IgG_1 concentration versus measured mouse IgG_1 concentration.

The line obtained had a slope of 0.920 with a correlation coefficient of 0.999.

Precision

Intra-assay precision was determined by taking samples containing low, medium and high concentrations of mouse IgG_1 and running these samples multiple times (n=19) in the same assay. Inter-assay precision was determined by measuring three samples with low, medium and high concentrations of mouse IgG_1 in multiple assays run over 9 days (n=16).
The precision numbers listed below represent the percent coefficient of variation for the concentrations of mouse IgG_1 determined in these assays as calculated by a 4 parameter logistic curve fitting program.

	m IgG_1 (ng/mL)	Intra-assay %CV	Inter-assay %CV
Low	49.9	1.2	
Medium	99.6	2.7	
High	160.4	3.6	
Low	48.5		3.1
Medium	98.3		4.4
High	148.7		8.1

Cross Reactivities

The IgG_1 (mouse) ELISA kit is specific for mouse IgG_1. It has a cross-reactivity of 0.9% with rat IgG_1 and 0.21% with mouse IgG_{2b}. It has less than 0.01% cross-reactivity with human IgG_1 and the following mouse proteins: IgG_{2a}, IgG_3, and IgM.

REFERENCES

1. T. Chard, "An Introduction to Radioimmunoassay & Related Techniques, 4th Edition", (1990) Amsterdam: Elsevier.
2. P. Tijssen, "Practice & Theory of Enzyme Immunoassays", (1985) Amsterdam: Elsevier.
3. P. Parham, **"**The Immune System**"**, (2000) New York: Garland Publishing.
4. National Committee for Clinical Laboratory Standards Evaluation Protocols, SC1, (1989) Villanova, PA: NCCLS.

NOTES

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