INSTRUCTIONS

Easy-Titer IgG Assay Kits

23300 23305 23310 23325

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Number	Description
23300	**Easy-Titer Mouse IgG Assay Kit**
23305	**Easy-Titer Rabbit IgG Assay Kit**
23310	**Easy-Titer Human IgG (H+L) Assay Kit**
23325	**Easy-Titer Human IgG (gamma chain) Assay Kit**

Kit Contents:

Kits include sufficient components for 96 tests. An IgG standard must be purchased separately.

Anti-Mouse, Anti-Rabbit, Anti-Human IgG (H+L) or Anti-Human IgG (gamma chain) Sensitized Polystyrene Beads, 2mL, stabilized in a phosphate buffer, pH 7.4, containing bovine serum albumin and 0.1% sodium azide

Dilution Buffer, 30mL, contains 0.1% sodium azide

Blocking Reagent, 15mL, contains 0.1% sodium azide

Storage: Upon receipt store all kit components at 4°C. Kits are shipped at ambient temperature. Do not freeze kit components.

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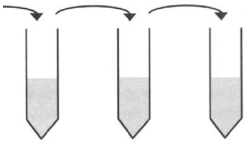
Introduction

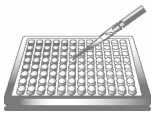
The Thermo Scientific™ Easy-Titer™ IgG Assay Kits are simple, mix-and-read assays that allow quick and accurate determination of concentrations from 15-300ng/mL of IgG. The assay procedure uses monodispersed polystyrene beads that are coated with anti-IgG antibodies and absorb light at 340 and 405 nm. When the beads are mixed with a sample containing IgG, they aggregate, causing decreased absorption of light and, therefore, low IgG concentrations yield high absorbance values and high IgG concentrations yield low absorbance values. The decrease in absorption is proportional to IgG concentration and a standard curve can be generated to accurately quantify levels of IgG in serum, ascites or cell culture supernatant samples. The Easy-Titer IgG Assay Kits feature a simple procedure with highly reproducible results.

Procedure Summary

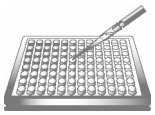
1. Suspend Anti- IgG Sensitized Beads.



2. Prepare standards and sample(s).

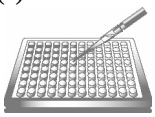


3. Add 20µL of the beads to the wells.



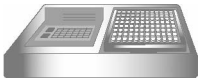
4. Add 20µL of the sample to the wells.

5. Incubate on a plate mixer for 5 minutes at room temperature.



6. Add 100µL of Blocking Buffer to the wells.

7. Mix plate for 5 minutes on a plate mixer.



8. Measure the absorbance at 405nm or 340nm. Plot standard curve to determine sample concentration.

Important Product Information

- Human bodily fluid must be handled and treated as a potentially infectious agent. Please adhere to local regulations for handling and disposal of infectious waste.
- Equilibrate all kit reagents to room temperature before use.
- The anti IgG-sensitized beads may settle in the cap area during shipping and storage. Mix the sensitized beads end-over-end on a rotator for at least 10 minutes. Just before dispensing, vortex the beads vigorously for 60 seconds. The combination of end-over-end and vortex mixing will ensure that the beads are monodispersed.
- The working range of this assay is 15-300ng/mL of IgG. If sample concentration is unknown, make serial dilutions and assay each dilution.

  Note: Purified human immunoglobulin subclasses, such as IgG_1, IgG_2, IgG_3 and IgG_4, have a suggested working range from 7.8 to 250ng/mL.
- The Easy-Titer Human IgG (H+L) Assay Kit recognizes both the heavy and the light chains (kappa and lambda) of human IgG. The Easy-Titer Human IgG (gamma chain) Assay Kit is selective for only the gamma chain (i.e., intact Fc) of human IgG.
- Absorbance values will often vary with each specific kit lot and with each assay performed. Always prepare a new IgG standard curve for each assay.
- The Anti-Mouse Sensitized Polystyrene Beads in the Easy-Titer Mouse IgG Assay Kit cross-react with rat IgG.

Additional Materials Required

- Standard IgG preparation to use in generating a standard curve (e.g., Mouse IgG, Product No. 31903; Rabbit IgG, Product No. 31235; or Human IgG, Product No. 31154)
- Pipettors to accurately deliver 10-1000µL
- 96-well microplate(s) (e.g., Product No. 15031 or 15041)
- Microplate mixer (vigorous mixing capability is required)
- Microplate reader that can measure absorbance at 405nm or 340nm
- Phosphate buffered saline (PBS; e.g., 0.1M phosphate, 0.15M NaCl; pH 7.2-7.4, Product No. 28372)

Easy-Titer IgG Assay Procedure

A. Sensitized Bead Preparation

Mix the Anti-IgG Sensitized Beads end-over-end on a rotator for at least 10 minutes. Just before dispensing, vortex the beads vigorously for 60 seconds.

B. IgG Standard Preparation

Dilute the chosen IgG standard to 100µg/mL with PBS. Then make additional dilutions with the supplied Dilution Buffer, as described in Table 1. The sample dilution scheme allows for multiple replicates of each standard concentration and may be scaled down.

Note: Do not use PBS to make the final dilution series. The Dilution Buffer must be used to make the final dilutions.

Table 1. Preparation of IgG standards.

Vial	Dilution Buffer Volume (µL	Volume and Source of IgG Standard	Final IgG Concentration (ng/mL)
A	995	5µL of 100µg/mL stock	500
B	500	500µL of Vial A	250
C	500	500µL of Vial B	125
D	500	500µL of Vial C	62.5
E	500	500µL of Vial D	31.2
F	500	500µL of Vial E	15.6

C. Sample Preparation

Samples must be at 15-300ng/mL for use in the assay. Dilute concentrated samples (Table 2) in PBS to a final estimated concentration of 100µg/mL. Then make a subsequent 1:200 to 1:1000 dilution with the Dilution Buffer supplied with the kit. Undiluted or slightly diluted PBS is not compatible with the assay.

Note: Be careful to conserve Dilution Buffer when diluting concentrated samples. For example, make final 1:200 dilutions by mixing 1µL of sample with 199µL of Dilution Buffer.

Table 2. Typical IgG concentration ranges in common sample types.

Normal Serum:	5.5- 22mg/mL
Ascites:	0.5-5.0mg/mL
Cell Culture Supernatant:	0.01-0.05mg/mL

Note: Both the Human IgG (H+L) Assay Kit and the Human IgG (gamma chain) Assay Kit may be used for the determination of human IgG levels in serum; however, do not use citrate- or EDTA-treated samples.

D. Assay Protocol

- Perform all steps at room temperature.
- The timing of the incubation steps is not critical; however, for best results perform all incubations for 2-10 minutes.
- To avoid error, assay samples in triplicate.
- Absorbance values will often vary with each specific kit lot and with each assay performed. Always prepare a new IgG standard curve for each assay.

1. Carefully pipette 20µL of the sensitized beads prepared in Section A into the appropriate number of wells in a 96-well microplate.

   Note: Work quickly to avoid settling of the sensitized beads. If dispensing the sensitized beads extends for longer than 60 seconds, recap the bottle and vortex beads for 5 seconds.

2. Carefully add 20µL of sample or standards (prepared in Sections B and C) into the appropriate wells containing beads.

3. Mix the microplate continuously on a plate mixer at a moderate-to-high speed setting for 5 minutes.

   Note: Vigorous mixing is critical to ensure adequate and thorough integration of the sensitized beads with the sample.

4. Add 100µL of the Blocking Buffer to each well. To avoid spills, reduce the plate mixer speed to a moderate speed setting and mix the microplate continuously for 5 minutes.
5. Before evaluating the plate, remove or burst all large bubbles. Measure the absorbance at either 405nm or 340nm.
6. Generate a standard curve and determine the sample concentration from the standard curve. Include the dilution factor for each sample when determining its starting IgG concentration. Most calculations can be easily performed with the software available with most ELISA plate readers.

E. Calculations

A standard curve (Figure 1) was generated using the data from Table 3. The IgG concentration is determined by interpolating between points on the curve. For example, a diluted sample yielded an absorbance of 0.715. Because the unknown has a value between the two standards 125 and 250ng/mL (Table 3), the linear interpolation equation is as follows: 125+125[(0.820-0.715)/(0.820-0.699)] = 233.5ng/mL. The sample was diluted 1:40,000 and, therefore, the IgG concentration in the original sample is 9.34mg/mL.

Note: A nonlinear regression analysis also may be performed on the entire curve. Please refer to the computer software manual for information concerning nonlinear regression.

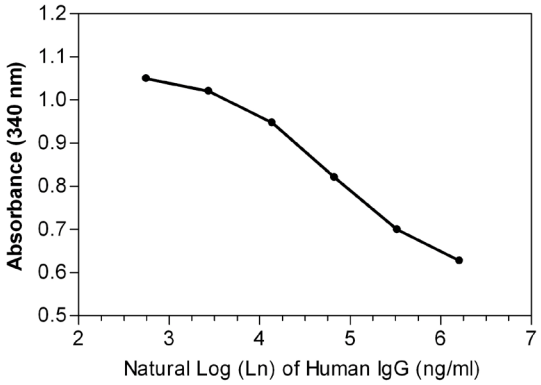


Figure 1. Standard curve for human IgG (suggested standard curve range: 15.6ng/mL to 500ng/mL). The data from Table 3 were used to plot the semi-log graph.

Table 3. Example assay standard curve values.

Standard (ng/mL)	Ln Standard (ng/mL)	Absorbance (340nm)
500.0	6.21	0.627
250.0	5.52	0.699
125.0	4.83	0.820
62.5	4.14	0.947
31.2	3.44	1.020
15.6	2.75	1.049

Troubleshooting

Problem	Possible Cause	Solution
Poor precision (high CVs) for all standards and samples	Inadequate mixing of diluted sample with the beads	Mix the plate vigorously for 5 minutes
	Inconsistent pipetting technique	Dispense the beads and diluted sample carefully
Poor precision for one sample or standard	Some of the wells contain bubbles	Burst bubbles with a gentle stream of air or with the tip of a pasteur pipette and measure the plate again
Low absorbance for the blank	Inadequate mixing of the beads	Mix the beads end-over-end for 10 minutes then vortex 10 seconds just before dispensing

Product References

Brown, M.A., *et al.* (2000). Identification and purification of vitamin K-dependent proteins and peptides with monoclonal antibodies specific for γ-carboxyglutamyl (Gla) residues. *J. Biol. Chem.* **275(26):**19795-802.

Cammarota, M., *et al*. (2003). Histamine activates tyrosine hydroxylase in bovine adrenal chromaffin cells through a pathway that involves ERK1/2 but not p38 or JNK. *J. Neurochem.* **84:**453-8.

Shan, G., *et al.* (2000). Isotope-labeled immunoassays without radiation waste. *PNAS* **97(6):**2445-9.

Current product instructions are available at *www.thermoscientific.com/pierce*. For a faxed copy, call 800-874-3723 or contact your local distributor.