Calculation of Results

Calculate the mean absorbance for each set of duplicate standards, controls and samples. Subtract the mean zero standard absorbance from each.

Plot the standard curve on log-log graph paper, with IgG_{2a} concentration on the x-axis and absorbance on the y-axis. Draw the best fit curve through the standard points.

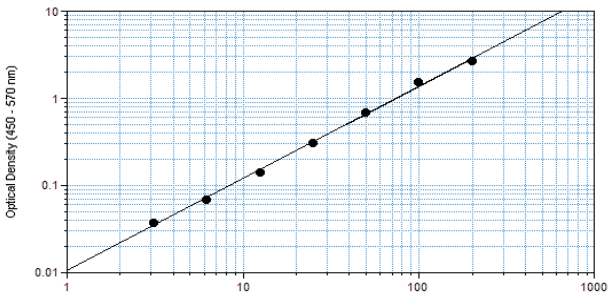
To determine the IgG_{2a} concentration of the unknowns, find the unknown's mean absorbance value on the y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the x-axis and read the IgG_{2a} concentration. If samples were diluted, multiply the IgG_{2a} concentration by the dilution factor.

Computer data reduction may also be employed, utilizing log-log regression analysis.

Typical Standard Curve

This standard curve is for demonstration only. A standard curve must be run with each assay.

BD OptEIA™ Mouse IgG_{2a} Set



Specificity

Cross Reactivity: The following immunoglobulins were tested at 10 µg/ml for potential cross-reactivity in the BD OptEIA™ mouse IgG_{2a} ELISA Set.

No significant cross-reactivities were identified (values ≥ 3.1 ng/ml).

Mouse Immunoglobulins:
IgG_1 (κ), IgG_1 (λ), IgG_{2b} (κ), IgG_{2b} (λ), IgG_3 (κ), IgG_3 (λ), IgM (κ), IgM (λ), IgA (κ), IgA (λ), IgE (κ)

Rat Immunoglobulins
IgG_1 (κ), IgG_1 (λ), IgG_{2a} (κ), IgG_{2a} (λ), IgG_{2b} (κ), IgG_{2b} (λ), IgG_{2c} (κ), IgM (κ), IgM (λ), IgA (κ), IgE (κ)

Serum samples from different mouse species (BALB/c, NZB, NZBWF1, and BXSB), immunized or unimmunized, were tested in this assay. The measured IgG_{2a} concentrations ranged from 0.16 mg/ml to 5.46 mg/ml. No detectable IgG_{2a} was found in SCID mouse (RAG-/-) serum.

Immunoglobulin Allotypes

Researchers should be aware that slight differences with the measurements of IgG_{2a} levels may occur across different species and/or allotypes. The BD OptEIA™ mouse IgG_{2a} Set is known to have different affinities for IgG_{2a} from mice of the b allotype as compared with the a allotype. It recognizes a allotype IgG_{2a} approximately 8.1 time better than b allotype IgG_{2a}. Because a purified monoclonal IgG_{2a} antibody of the a allotype is used for the standard in this assay, a conversion factor of 8.1 should be used to quantitate b allotype IgG_{2a} levels. For example, a group of C57BL/6 (b allotype) mouse serum samples were tested using this assay and showed a concentration range of 1.04–7.84 µg/ml, the actual range should be of 8.4–63.5 µg/ml after the conversion.

Standardization

This immunoassay is calibrated against purified mouse IgG_{2a}.

Assay Optimization

1. BD OptEIA™ Sets allow flexible assay design to fit individual laboratory needs. To design an immunoassay with different sensitivity and dynamic range, the following parameters can be varied: Capture, Detection Antibody titers, Incubation time, Incubation temperature, Assay Diluent formulation, Buffer pH, ionic strength, protein concentration, Type of substrate, Washing technique (i.e., number of wash repetitions and soak times)
2. "Typical Standard Curve" and 20-plate yield were obtained in the BD Biosciences Pharmingen laboratory, using the recommended procedure and manual plate washing.

Troubleshooting

	Possible Source	Corrective Action
Poor Precision		
	• Inadequate washing/ aspiration of wells	• Check function of washing system
	• Inadequate mixing of reagents	• Ensure adequate mixing
	• Imprecise/ inaccurate pipetting	• Check/ calibrate pipettes
	• Incomplete sealing of plate	• Ensure complete seal on plate
Poor Standard Curve		
	• Improper standard handling/ dilution standards	• Ensure correct preparation, storage of
	• Incomplete washing/ aspiration of wells	• Check function of washing system
	• Imprecise/ inaccurate pipetting	• Check/ calibrate pipettes
	• Improper buffer/ diluent used	• Check buffer/ diluent preparation, pH
Low Absorbances		
	• Inadequate reagent volumes added to wells	• Check/ calibrate pipettes
	• Incorrect incubation times/ temperature	• Ensure sufficient incubation times/reagents warmed to RT
	• Incorrect antibody titration Detector preparation	• Check Capture Ab and Working
	• Improper buffer/ diluent used	• Check buffer/ diluent preparation, pH
	• Overly high wash/aspiration pressure from automated plate-washer	• Utilize manual washing

Limitations of the Procedure

• Samples that generate absorbance values higher than the standard curve should be diluted with Standard Diluent and re-assayed.
• Interference by drug metabolites, soluble receptors, or other binding proteins in specimens has not been thoroughly investigated. The possibility of interference cannot be excluded.
• BD OptEIA™ Sets are intended for use as an integral unit. Do not mix reagents from different Set batches. Reagents from other manufacturers are not recommended for use in this Set.

BD OptEIA™

Technical Data Sheet

Mouse IgG_{2a} ELISA Set

Cat. No. 552576

Materials Provided

The OptEIA™ Set for mouse IgG_{2a} contains the components necessary to develop enzyme-linked immunosorbent assays (ELISA) for natural or purified mouse IgG_{2a} in serum, plasma, and cell culture supernatants. Sufficient materials are provided to yield approximately 20 plates of 96-wells if the recommended storage, materials, buffer preparation, and assay procedure are followed as specified in this package.

Capture Antibody

Anti-Mouse IgG_{2a} monoclonal antibody

Detection Antibody

Biotinylated Anti-Mouse IgG_{2a} monoclonal antibody

Enzyme Reagent

Streptavidin-horseradish peroxidase conjugate (SAv-HRP)

Standards

Purified mouse IgG_{2a}, lyophilized

Instruction / Analysis Certificate

(lot-specific)

United States
877.232.8995

Canada
866.979.9408

Europe
32.2.400.98.95

Japan
0120.8555.90

Asia/Pacific
65.6861.0633

Latin America/Caribbean
55.11.5185.9995

BD Biosciences

BD Biosciences Pharmingen
10975 Torreyana Road
San Diego, CA 92121
Customer/Technical Service
Tel 877.232.8995 (US)
Fax 858.812.8888
www.bdbiosciences.com

Recommended buffers, solutions

Note: Do not use sodium azide in these preparations. Sodium azide inactivates the horseradish peroxidase enzyme.

The BD OptEIA™ Reagent Set B (Cat. No 550534) containing Coating Buffer, Assay Diluent, Substrate Reagents A and B, Stop Solution and 20X Wash Buffer Concentrate is recommended.

1. **Coating Buffer** - 0.1 M Sodium Carbonate, pH 9.5
   7.13 g $NaHCO_3$, 1.59 g Na_2CO_3; q.s. to 1.0 L; pH to 9.5 with 10N NaOH.
   Freshly prepare or use within 7 days of preparation, stored at 2-8°C.
2. **Assay Diluent**- PBS* with 10% FBS#, pH 7.0. The BD Pharmingen™ Assay Diluent (Cat. No. 555213) is recommended.
   *Phosphate-Buffered Saline: 80.0 g NaCl, 11.6 g Na_2HPO_4, 2.0 g KH_2PO_4, 2.0 g KCL, q.s. to 10 L; pH to 7.0.
   #Fetal Bovine Serum: Hyclone Cat. No. SH30088 (heat-inactivated) recommended.
   Freshly prepare or use within 3 days of preparation, with 2-8°C storage.
3. **Wash Buffer** - PBS* with 0.05% Tween-20. Freshly prepare or use within 3 days of preparation, stored at 2-8°C.
4. **Substrate Solution** - Tetramethylbenzidine (TMB) and Hydrogen Peroxide. The BD Pharmingen™ TMB Substrate Reagent Set (Cat. No. 555214) is recommended.
5. **Stop Solution** - 1 M H_3PO_4 or 2 N H_2SO_4

Additional Materials Required

1. 96-well Nunc-Immuno™ polystyrene Maxisorp ELISA flat bottom plates (ThermoFisher Scientific Cat. No. 442404) are recommended
2. Microplate reader capable of measuring absorbance at 450 nm
3. Precision pipettes
4. Graduated cylinder, one liter
5. Deionized or distilled water
6. Wash bottle or automated washer
7. Log-log graph paper or automated data reduction
8. Tubes to prepare standard dilutions
9. Laboratory timer
10. Plate sealers or parafilm

Storage Information

1. Store unopened reagents at 2-8°C. Do not use reagents after expiration date, or if turbidity is evident.
2. Before use, bring all reagents to room temperature (18-25°C). Immediately after use, return to proper storage conditions.
3. Lyophilized standards are stable until expiration date. See below for reconstituted standard storage information.

Specimen Collection and Handling

Specimens should be clear, non-hemolyzed and non-lipemic.

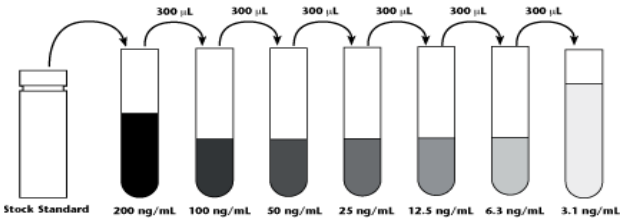
Cell culture supernatants: Remove any particulate material by centrifugation and assay immediately or store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.

Serum: Use a serum separator tube and allow samples to clot for 30 minutes, then centrifuge for 10 minutes at 1000 x g. Remove serum and assay immediately or store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

Plasma: Collect plasma using citrate, EDTA, or heparin as anticoagulant. Centrifuge for 10 minutes at 1000 x g within 30 minutes of collection. Assay immediately or store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

Standards Preparation and Handling

1. **Reconstitution:** After warming lyophilized standard to room temperature, carefully open vial to avoid loss of material. Reconstitute lyophilized standard with 1.0 mL of deionized water to yield a stock standard. Allow the standard to equilibrate for at least 15 minutes before making dilutions. Vortex gently to mix.
2. **Storage/ handling of reconstituted standard:** After reconstitution, **immediately** aliquot standard stock in polypropylene vials at 50 µl per vial and freeze at -80°C for up to 6 months. If necessary, store at 2-8° C for up to 8 hours prior to aliquotting/freezing. Do not leave reconstituted standard at room temperature.
3. **Standards Preparation for Assay:**
   a. Prepare a 200 ng/mL standard from the stock standard. Vortex to mix. (See dilution instructions on Instruction/Analysis Certificate.)
   b. Add 300 µL Assay Diluent to 6 tubes. Label as 100 ng/mL, 50 ng/mL, 25 ng/mL, 12.5 ng/mL, 6.3 ng/mL, and 3.1 ng/mL.
   c. Perform serial dilutions by adding 300 µL of each standard to the next tube and vortexing between each transfer. Assay Diluent serves as the zero standard (0 pg/mL).



Serial dilutions within the plate may also be performed by pipetting 100 µL of Assay Diluent into each standard well except the highest (200 ng/mL), then adding 100 µL of the 200 ng/mL standard to both that well and the 100 ng/mL well, mixing the well contents by rinsing the pipette tip, and adding 100 µL of the 100 ng/mL standard to the 50 ng/mL well. Continue these dilutions to the 3.1 ng/mL standard well, out of which the extra 100 µL should be discarded.

Warnings and Precautions

1. Reagents which contain preservatives may be toxic if ingested, inhaled, or in contact with skin.
2. Handle all serum and plasma specimens in accordance with NCCLS guidelines for preventing transmission of blood-borne infections.
3. Capture Antibody contains < 0.1% sodium azide. Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
4. Detection Antibody contains BSA and ProClin®-150 as a preservative.
5. Enzyme Reagent contains BSA and ProClin®-150 as a preservative.
6. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
7. ***Warning:*** Recombinant Mouse IgG_{2a} lyophilized standard (component 51-9000076) contains 0.02% (w/w) and Detection Antibody Biotin Anti-Mouse IgG2a (component 51-9000075) contains 0.002% (w/w) of a CMIT/MIT mixture (3:1), which is a mixture of: 5-chloro-2-methyl-4-isothiazolin-3-one [EC No 247-500-7] and 2-methyl-4-isothiazolin-3-one [EC No 220-239-6] (3:1).
   Hazard statements
   May cause an allergic skin reaction.
   Precautionary statements
   Wear protective gloves / eye protection.
   Wear protective clothing.
   Avoid breathing mist/vapours/spray.
   If skin irritation or rash occurs: Get medical advice/attention.
   IF ON SKIN: Wash with plenty of water.
   Dispose of contents/container in accordance with local/regional/national/international regulations.

Recommended Assay Procedure

1. Coat microwells with 100 µL per well of Capture Antibody diluted in Coating Buffer. For recommended antibody coating dilution, see lot-specific Instruction/Analysis Certificate. Seal plate and incubate overnight at 4° C.
2. Aspirate wells and wash 3 times with ≥ 300 µL/well Wash Buffer. After last wash, invert plate and blot on absorbent paper to remove any residual buffer.
3. Block plates with ≥ 200 µL/well Assay Diluent. Incubate at RT for 1 hour.
4. Aspirate/wash as in step 2.
5. Prepare standard and sample dilutions in Assay Diluent. See "Standards Preparation and Handling".
6. Pipette 100 µL of each standard, sample, and control into appropriate wells. Seal plate and incubate for 2 hours at RT.
7. Aspirate/ wash as in step 2, but with 5 total washes.
8. Add 100 µL of Detection Antibody diluted in Assay Diluent to each well. Seal plate and incubate for 1 hour at RT.
9. Aspirate/ wash as in step 2, but with 5 total washes.
10. Add 100 µL of Enzyme Reagent diluted in Assay Diluent to each well. Seal plate and incubate for 30 min at RT.
11. Aspirate/ wash as in step 2, but with 7 total washes. NOTE: In this final wash step, soak wells in wash buffer for 30 seconds to 1 minute for each wash.
12. Add 100 µL of Substrate Solution to each well. Incubate plate (without plate sealer) for 30 minutes at room temperature in the dark.
13. Add 50 µL of Stop Solution to each well.
14. Read absorbance at 450 nm within 30 minutes of stopping reaction. If wavelength correction is available, subtract absorbance at 570 nm from absorbance 450 nm.

Assay Procedure Summary

1. Add 100 µL diluted Capture Ab to each well.
   Incubate overnight at 4°C.
2. Aspirate and wash 3 times.
3. Block plates: 200 µL Assay Diluent to each well. Incubate 1 hr RT
4. Aspirate and wash 3 times.
5. Add 100 µL standard or sample to each well. Incubate 2 hr RT.
6. Aspirate and wash 5 times.
7. Add 100 µL diluted Detection Ab to each well. Incubate 1 hr RT.
8. Aspirate and wash 5 times.
9. Add 100 µL diluted Streptavidin-HRP to each well. Incubate 30 min RT.
10. Aspirate and wash 7 times (with 30 sec to 1 min soaks)
11. Add 100 µL Substrate Solution to each well. Incubate 30 min RT in dark
12. Add 50 µL Stop Solution to each well. Read at 450 nm within 30 min with λ correction 570 nm.

References

1. Emson C, et al. *J Exp Med.* 1998; 188: 399-404.
2. Kuhn R, et al. *Science.* 1991; 254: 707-710.
3. Cascalho M, et al. *Science.* 1996; 272: 1649-1652.
4. Stall A, et al. *Immunohistochemistry and Molecular Immunology.* 1996; 1: 27.1-27.16.