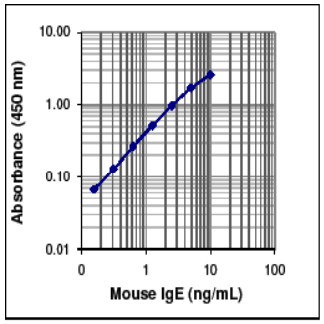
Calculation of Results

Plot the standard curve on log-log axis graph paper with cytokine concentration on the x-axis and absorbance on the y-axis. Draw a best fit line through the standard points. To determine the unknown cytokine concentrations in the samples, find the absorbance value of the unknown 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 corresponding cytokine concentration. If the samples were diluted, multiply by the appropriate dilution factor. The data is best calculated with computer-based curve-fitting software using a 5- or 4-parameter logistics curve-fitting algorithm. If a test sample's absorbance value falls outside the standard curve ranges, that test sample needs to be reanalyzed at a higher or lower dilution as appropriate.

Typical Data

Standard Curve: This standard curve was generated at BioLegend for demonstration purposes only. A standard curve must be run with each assay.



Performance Characteristics

Specificity: No cross reactivity was detected when this kit was used to analyze other mouse immunoglobulins and IgE from other species.

Troubleshooting

High Background:

- Background wells were contaminated.
- Matrix used had endogenous analyte.
- Plate was insufficiently washed.
- TMB Substrate Solution was contaminated.

No signal:

- Incorrect or no antibodies were added.
- Avidin-HRP was not added.
- Substrate solution was not added.
- Wash buffer contained sodium azide.

Low or poor signal for the standard curve:

- Standard was incompletely reconstituted or was stored improperly.
- Reagents were added to wells with incorrect concentrations.
- Plate was incubated with inappropriate temperature, timing or agitation.

Signal too high, standard curves saturated:

- Standard was reconstituted with less volume than required.
- One or more reagent incubation steps were too long.
- Plate was incubated with inappropriate temperature, timing, or agitation.

Sample readings out of range:

- Samples contain no or below detectable levels of analyte.
- Samples contain analyte concentrations greater than highest standard point.

High variations in samples and/or standards:

- Pipetting errors may have occurred.
- Plate washing was inadequate or nonuniform.
- Samples were not homogenous.
- Samples or standard wells were contaminated.

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BioLegend
The path to legendary discovery™

Mouse IgE

ELISA MAX™ Standard Sets

Cat. No. 432401 (5 plates)
432402 (10 plates)
432403 (20 plates)

BioLegend's ELISA MAX™ Standard Sets contain the capture and detection antibodies, standard, and Avidin-HRP required for the accurate quantification of natural and recombinant mouse IgE. These sets are cost-effective and designed for experienced ELISA users. Optimization of reagent concentrations and assay conditions may be required.

It is highly recommended that the instruction sheet be read in its entirety before using this product. Use the recommended assay protocol, microwell plates, buffers, diluent, and substrate solution to obtain desired assay results. Do not use this set beyond the expiration date.

Materials Provided

1. Mouse IgE ELISA MAX™ Capture Antibody (200X)
2. Mouse IgE ELISA MAX™ Detection Antibody (200X)
3. Mouse IgE Standard
4. Avidin-HRP (1000X)
5. Instruction Sheet
6. Lot-Specific Instruction/ Analysis Certificate

Principle of the Test

BioLegend's ELISA MAX™ Standard Set is a sandwich Enzyme-Linked Immunosorbent Assay (ELISA).

For research purposes only. Not for use in diagnostic or therapeutic procedures.

Rev. 120111

Materials to be Provided by the End-User

- Microwell plates: 96-well Nunc MaxiSorp™ is recommended.
- A microplate reader capable of measuring absorbance at 450 nm
- Adjustable pipettes to measure volumes ranging from 2 µL to 1 mL
- Deionized (DI) water
- Coating Buffer: 8.4 g $NaHCO_3$, 3.56 g Na_2CO_3, add DI H_2O to 1.0 L, pH to 9.5 (BioLegend Cat. No. 421701 is recommended.)
- Assay Diluent: 10% Fetal Bovine Serum or 1% BSA in Phosphate-Buffered Saline (PBS) (BioLegend Cat. No. 421203 is recommended.)
- PBS: 8.0 g NaCl, 1.16 g Na_2HPO_4, 0.2 g KH_2PO_4, 0.2 g KCl, add DI water to 1.0 L, pH to 7.4
- Wash Buffer: Phosphate-Buffered Saline (PBS) + 0.05% Tween-20 (BioLegend Cat. No. 421601 is recommended.)
- Wash bottle or automated microplate washer
- TMB Substrate Solution – BioLegend Cat. No. 421101 is recommended.
- Stop Solution (2 N H_2SO_4)
- Log-Log graph paper or software for data analysis
- Tubes to prepare standard dilutions
- Timer
- Absorbent paper

Storage Information

- Store kit components at 4°C.
- After reconstitution of the lyophilized standard with Assay Diluent, aliquot into polypropylene vials and store at -70°C for up to one month. Avoid repeated freeze/thaw cycles.
- Prior to use, bring all components to room temperature (18-25°C). Upon assay completion return all components to appropriate storage conditions.

Health Hazard Warnings

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS online for details (www.biolegend.com/support/#msds).
2. TMB substrate solution is harmful if ingested. Additionally, avoid skin, eye or clothing contact.
3. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum and/or plasma in accordance with NCCLS regulations.

Specimen Collection and Handling

Cell Culture Supernatant: If necessary, centrifuge to remove debris prior to analysis. Samples can be stored at < -20°C. Avoid repeated freeze/thaw cycles.

Serum: Use a serum separator tube and allow clotting for at least 30 minutes, then centrifuge for 10 minutes at 1,000 X *g*. Remove serum layer and assay immediately or store serum samples at < -20°C. Avoid repeated freeze/thaw cycles. Serum specimens should be clear and non-hemolyzed.

Plasma: Collect blood sample in a citrate, heparin or EDTA containing tube. Centrifuge for 10 minutes at 1,000 X *g* within 30 minutes of collection. Assay immediately or store plasma samples at < -20°C. Avoid repeated freeze/thaw cycles. Plasma specimens should be clear and non-hemolyzed.

Reagent Preparation

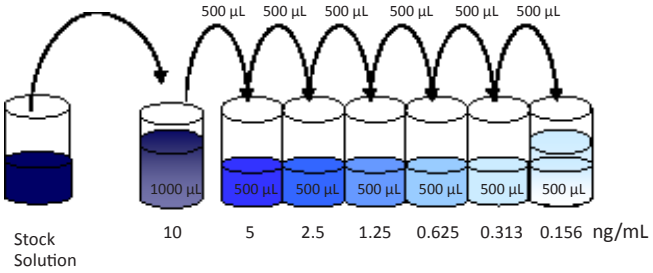
Do not mix reagents from different sets or lots. Avidin-HRP, Mouse IgE Standard, and/or antibodies from different manufacturers should not be used with this set. All reagents should be diluted immediately prior to use.

1. Dilute the pre-titrated Capture Antibody 1:200 in Coating Buffer. For one plate, dilute 60 µL Capture Antibody in 11.94 mL Coating Buffer.
2. Lyophilized vial is under vacuum pressure. Reconstitute lyophilized standard with 0.2 mL of Assay Diluent. Allow the reconstituted standard to sit for 15 minutes at room temperature, then mix gently prior to making dilutions.
3. Prior to use, prepare 1,000 µL of the top standard at a concentration of 10 ng/mL from stock solution in Assay Diluent (**refer to Lot-Specific Instruction/Analysis Certificate**).
4. Dilute the pre-titrated Biotinylated Detection Antibody 1:200 in Assay Diluent. For one plate, dilute 60 µL Detection Antibody in 11.94 mL Assay Diluent.
5. Dilute Avidin-HRP 1:1000 in Assay Diluent. For one plate, dilute 12 µL Avidin-HRP in 11.99 mL Assay Diluent.
6. Prepare all other reagents required for the assay including TMB Substrate Solution. Refer to reagent description in the section **"Materials to be Provided by the End-User"**.

Assay Procedure

Do not use sodium azide in any solutions as it inhibits the activity of the horseradish-peroxidase enzyme.

1. One day prior to running the ELISA, dilute Capture Antibody in Coating Buffer. Add 100 µL of this Capture Antibody solution to all wells of a 96-well plate provided in the set. Seal plate and incubate overnight at 4°C.
2. Bring all reagents to room temperature (RT) prior to use. It is strongly recommended that all standards and samples be run in duplicate or triplicate. A standard curve is required for each assay.
3. Wash plate 4 times with at least 300 µL Wash Buffer per well and blot residual buffer by firmly tapping plate upside down on absorbent paper. **All subsequent washes should be performed similarly.**
4. To block non-specific binding and reduce background, add 200 µL Assay Diluent per well.
5. Seal plate and incubate at RT for 1 hour with shaking at 200 rpm on a plate shaker.
6. While plate is being blocked, prepare the appropriate sample dilutions (if necessary) and standards.
7. Prepare 1,000 µL of top standard at 10 ng/mL in Assay Diluent (refer to **Reagent Preparation**). Perform six two-fold serial dilutions of the 10 ng/mL top standard with Assay Diluent in separate tubes. After diluting, the mouse IgE standard concentrations are 10 ng/mL, 5 ng/mL, 2.5 ng/mL, 1.25 ng/mL, 0.625 ng/mL, 0.313 ng/mL and 0.156 ng/mL, respectively. Assay Diluent serves as the zero standard (0 pg/mL).



8. Wash plate 4 times with Wash Buffer.
9. Add 100 µL/well of standards or samples to the appropriate wells. If dilution is required, samples should be diluted in Assay Diluent before adding to the wells.
10. Seal plate and incubate at RT for 2 hours with shaking.
11. Wash plate 4 times with Wash Buffer.
12. Add 100 µL of diluted Detection Antibody solution to each well, seal plate and incubate at RT for 1 hour with shaking.
13. Wash plate 4 times with Wash Buffer.
14. Add 100 µL of diluted Avidin-HRP solution to each well.
15. Seal plate and incubate at RT for 30 minutes with shaking.
16. Wash plate 5 times with Wash Buffer. For this final wash, soak wells in Wash Buffer for 30 seconds to 1 minute for each wash. This will help minimize background.
17. Add 100 µL of TMB Substrate Solution and incubate **in the dark for 20-30 minutes or until the desired color develops*.** Positive wells should turn blue in color. It is not necessary to seal the plate during this step.
18. Stop reaction by adding 100 µL of Stop Solution to each well. Positive wells should turn from blue to yellow.
19. Read absorbance at 450 nm within 30 minutes. If the reader can read at 570 nm, the absorbance at 570 nm can be subtracted from the absorbance at 450 nm.

***Optimal substrate incubation time depends on laboratory conditions and the optical linear ranges of ELISA plate readers.**

Assay Procedure Summary

Day 1

Add 100 µL diluted Capture Antibody solution to each well, incubate overnight at 4° C

Day 2

1. Wash plate 4 times
2. Add 200 µL Assay Diluent to block, incubate at room temperature for 1 hour with shaking
3. Wash plate 4 times
4. Add diluted standards and samples to the appropriate wells, incubate at room temperature for 2 hours with shaking
5. Wash plate 4 times
6. Add 100 µL diluted Detection Antibody solution to each well, incubate at room temperature for 1 hour with shaking
7. Wash plate 4 times
8. Add 100 µL diluted Avidin-HRP solution to each well, incubate at room temperature for 30 minutes with shaking
9. Wash plate 5 times, soaking for 30 seconds to 1 minute per wash
10. Add 100 µL of TMB Substrate Solution to each well, incubate in the dark for 20-30 minutes or until the desired color develops
11. Add 100 µL Stop Solution to each well
12. Read absorbance at 450 nm and 570 nm

For more information about BioLegend ELISA MAX™ Sets and LEGEND MAX™ ELISA Kits with precoated plates, visit **www.biolegend.com.**