mdbioproducts.

MonELISA® Series
Mouse OVA-IgE ELISA

Catalog Number M036005N

For the quantitative determination of OVA-IgE in mouse serum and cell culture supernate samples.

For research use only.

PRINCIPLE OF THE ASSAY

ELISA or Enzyme-linked Immunosorbent Assay is a colorimetric based immunoassay utilizing a capture antibody and a detection antibody to provide a unique and powerful assay system. Antibody/antigen reactions take place on the suface of microplate wells that have been previously coated with a monoclonal antibody to mouse IgE heavy chain. Biotinylated ovalbumin and streptavidin-peroxidase, in the presence of substrate quantifies the analyte bound.

KIT COMPONENTS

OVA-IgE Microplate (Part PL30019) - The plate contains 12 x 8 strips coated with anti-IgE heavy chain monoclonal antibody. The strips are ready to use.

OVA-IgE Standard (Part ST30020) - 1 vial (2500 ng/mL) of OVA-IgE in a protein buffer.

Conjugate Concentrate (Part BT30018) - 1 vial of a 100-fold concentrated biotinylated ovalbumin in a stabilizing buffer. Dilute in buffer provided in Ancillary Reagent Kit, Catalog Number M036080.

Streptavidin-HRP Concentrate (Part HP30006) - 1 vial of 100-fold concentrated streptavidin-HRP in a stabilizing buffer. Dilute in buffer provided in Ancillary Reagent Kit, Catalog Number M036080.

Plate Sealer - 2 adhesive strips.

THESE ASSAY COMPONENTS ARE TO BE USED WITH
5-PLATE ANCILLARY REAGENT KIT, CATALOG NUMBER M036080

SUPPLIES REQUIRED BUT NOT PROVIDED

- Pipettes or pipetting equipment with disposable polypropylene tips
- Glass measuring cylinders
- Distilled or deionized water
- Horizontal orbital microplate reader
- Squirt bottle or automated microplate washer
- Microplate reader capable of measuring at 450 nm
- Orbital shaker

PRECAUTIONS

Stop Solution consists of diluted sulfuric acid. Wear eye, hand, face, and clothing protection when using these materials. Avoid contact with skin and eyes. In case of contact wash immediately with water. All chemicals should be considered as being potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice.

The Assay Diluent contains sodium azide which may react with lead and copper plumbing to form explosive metallic azides. Flush with large volumes of water during disposal.

- For research use only. Not for internal or external use in humans or animals.
- This kit contains no material of human origin.
- For the handling of blood, (serum), we recommend that precautions should be observed.
- Please refer to HHS Publication no. (CDC) 88-8395 or corresponding local/ national guidelines on laboratory safety procedures.

CRITICAL PARAMETERS

- Allow samples and all reagents to equilibrate to room temperature (22 – 25 °C) prior to performing the assay. This is especially important for the TMB Substrate.
- Adhere to recommended incubation temperatures in the protocol as variations may cause inconsistent or poor assay results.
- It is essential that all wells are washed thoroughly and uniformly. When washing is done by hand, use a squeeze bottle and ensure that all wells are completely filled and emptied at each step.
- Use only reagents from the same lot for each assay. This is especially important when running more than one plate per sample group.
- A separate standard curve must be run on each plate.
- Reagents can easily be trapped in the caps of small microfuge tubes. It is recommended to briefly centrifuge these reagents before use.
- Mix all reagents thoroughly prior to use, but avoid foaming!
- Keep the wells sealed with the plate sealer except when adding reagents and during reading.
- Any variation in the protocol can cause variation in binding!
- The kit should not be used beyond the expiration date on the kit label.
- The values obtained by the samples should be within the standard range. If this is not the case, dilute the sample and repeat the assay.
- We take great care to ensure that this product is suitable for all validated sample types, as designated in this manual. Other sample types may be tested and validated by the user.

SAMPLE COLLECTION & STORAGE

Cell Culture Supernates- Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20 °C.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at approximately 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C.

SAMPLE PREPARATION

Serum and Cell Culture Supernate samples require at least a 10-fold dilution into Assay Diluent.

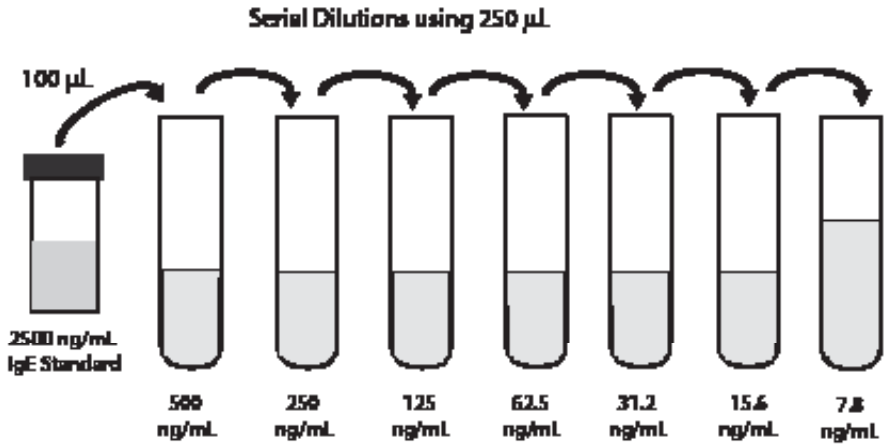
REAGENT PREPARATION

Bring all reagents to room temperature (22 - 25 °C) before use. Reagents can easily be trapped in the caps of small microfuge tubes. It is recommended to briefly centrifuge these reagents before use.

Wash Buffer (1X) - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 50 mL of Wash Buffer Concentrate into 450 mL of deionized water to prepare 500 mL of Wash Buffer (1X). Store for up to 30 days at 2 - 8 °C.

Conjugate (1X) - Dilute 115 µL of Conjugate Concentrate into 11.5 mL of Conjugate diluent. Store diluted conjugate for up to 30 days at -20 ºC.

Standards - Label 7 standard tubes as shown below. Pipette 400 µL Assay Diluent into the 500 ng/mL standard tube and 250 µL Assay Diluent into the remaining tubes. Use the 2500 ng/mL standard to produce a dilution series (see below). The 500 ng/mL standard serves as the high standard and Assay Diluent serves as the zero (0 ng/mL) standard.



Streptavidin-HRP (1X) - **Prepare within 30 minutes of use and keep protected from light**. Add 115 µL Streptavidin-HRP Concentrate to 11.5 mL of HRP Diluent. Prepare fresh Streptavidin-HRP (1X) for each assay. If running less than a full plate, prepare only the amount needed.

ASSAY PROTOCOL

Read the entire protocol before beginning the assay. It is recommended that all standards and samples be assayed in duplicate. Reagents can easily be trapped in the caps of small microfuge tubes. It is recommended to briefly centrifuge these reagents before use. *Note: Reagents and samples may require specific handling temperatures and need preparation prior to the assay. See the Reagent and Sample Preparation sections before proceeding*.

1. Prepare all reagents and samples as described in the previous sections.
2. Remove any excess microplate strips from the plate frame and return them to the foil pouch containing the desiccant pack.
3. Pipette 100 µL of standard or sample in duplicate into the wells using a clean pipette tip for each standard or sample. Cover with the plate sealer provided and incubate for 1 hour at room temperature (22 - 25 °C) on an orbital shaker (600 - 750 rpms).
4. Aspirate and wash the wells 4 times with 200 µL per well of Wash Buffer (1X). Take care that all wells are filled and emptied at each wash. Blot the plate on paper towels to remove residual fluid from the plate.
5. Add 100 µL of Conjugate (1X) into each well. Cover with the plate sealer provided and incubate for 30 minutes at room temperature on the shaker.
6. Aspirate and wash the wells 4 times with 200 µL per well of Wash Buffer (1X). Take care that all wells are filled and emptied at each wash. Blot the plate on paper towels to remove residual fluid from the plate.
7. Add 100 µL of diluted Streptavidin-HRP to each well. Incubate at room temperature for 30 minutes **without shaking. Protect from light.**
8. Aspirate and wash the wells 4 times with 200 µL per well of Wash Buffer (1X). Take care that all wells are filled and emptied at each wash. Blot the plate on paper towels to remove residual fluid from the plate.
9. Add 100 µL Substrate to each well and incubate for 15 minutes at room temperature. **Protect from light.**
10. Stop the reaction by adding 100 µL of Stop Solution to each well. Gently tap the side of the plate to ensure thorough mixing.
11. Read the plate at 450 nm.

SUMMARY

Prepare reagents and samples as previously described.

Pipette 100 µL Standard or diluted sample in duplicate into the wells. Incubate 1 hr at RT (22 - 25 °C) on shaker.

Aspirate and wash 4 times.

Add 100 µL of Conjugate (1X) to each well. Incubate 30 min. at RT on shaker.

Aspirate and wash 4 times.

Add 100 µL of diluted Streptavidin-HRP to each well. Incubate 30 min. at RT without shaking. Protect from light.

Aspirate and wash 4 times.

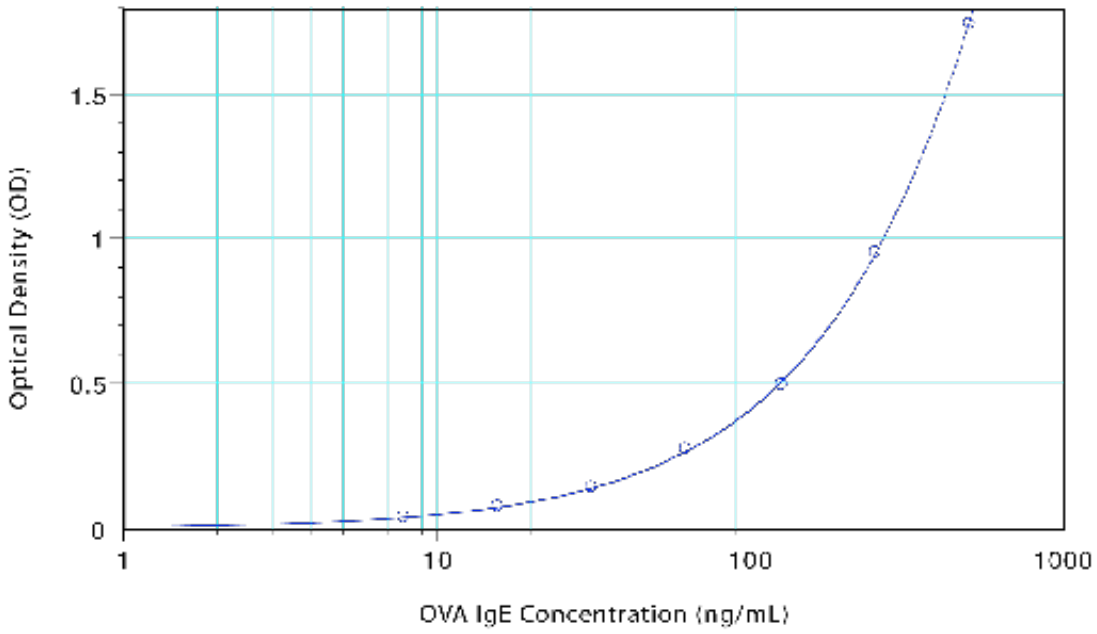
Add 100 µL of Substrate to each well. Incubate 15 min. at RT. Protect from light.

Add 100 µL of Stop Solution to each well. Read at 450 nm

CALCULATION OF RESULTS

Average the duplicate readings for each standard and sample and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the OVA-IgE concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

This standard curve is provided for demonstration only. A standard curve should be generated with each set of samples assayed.



PERFORMANCE CHARACTERISTICS

Sensitivity

Sensitivity is defined as the minimal detectable dose determined by adding two standard deviations of the mean optical density value for twenty replicates of the zero standard and calculating the corresponding concentration. The sensitivity of the OVA-IgE ELISA is typically less than 3.8 ng/mL.

Reproducibility

Intra-assay Precision (Precision within an assay) - The intra-assay precision was measured by assaying three control samples 20 times on one plate.

Inter-assay Precision (Precision between assays) - The inter-assay precision was assessed by repeated measurements of three control samples in 20 successive assays with multiple users.

	Intra-assay Precision			Inter-assay Precision		
Control	1	2	3	1	2	3
Mean (ng/mL)	15.6	108.5	302.6	15.1	100.2	278.3
Standard Deviation	1.55	7.0	15.5	1.5	9.5	20.3
CV (%)	9.8	6.7	5.3	9.8	9.5	7.3

LINEARITY

Natural mouse IgE samples were diluted 100-fold in Assay Diluent and serially diluted down to 1:16.

Species	Average	Range
Mouse	104%	95 - 120%

RECOVERY

Natural mouse IgE samples were spiked into media, diluted 100-fold in Assay Diluent and serially diluted down to 1:16. The average recovery of the assay is 104%.

SPECIFICITY

Mouse IgG was evaluated in the assay at different concentrations. The cross-reactivity of IgG is less than 0.01%.

TROUBLESHOOTING

Problem	Recommendation
Low Absorbance	· Check reagents for proper storage. · Control expiration date. · Check preparation of reagents. · Control incubation times and temperature. · Check reader wavelength.
High Absorbance/high zero standard value	· Check preparation of reagents. · Control incubation times and temperature. · Equilibrate ELISA reagents to room temperature (22 - 25 °C). · Ensure that every well of the ELISA plate is completely filled and emptied at every wash step. · Check that plates are blotted on tissue paper after washing.
Flat curve/poor reproduciblity	· Check reagents for proper storage. · Control expiration date. · Check preparation of working standards. · Check incubation times and temperatures. · Use separate reservoirs for pipetting different solutions with multichanned pipettes. Always use new pipette tips. · Check pipette calibration. · Ensure efficient washing procedure.

NOTES

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