mdbioproducts.

MonELISA® Series
Murine Total IgE Assay

Catalog Number M036023

For the quantitative determination of IgE in murine serum/plasma samples

For research use only.

INTRODUCTION

IgE is the least abundant isotype but has the capability of triggering powerful immune responses by binding to Fc receptors on the surface of cells such as mast cells, basophils, eosinophils, monocytes, macrophages and platelets.

IgE is well known for its involvement in eliciting an allergic or asthmatic response. After sensitization with an allergan, through T and B cell signalling stimulates IgE production. IgE is released into the bloodstream where it attaches to receptors on cells such as mast cells and basophils. These cells are then activated upon cross-linking of the IgE with the antigen initiating the allergic cascade.

KIT COMPONENTS

IgE specific Microplate - The plate contains 12 x 8 strips coated with antibody to species-specific IgE. The strips are ready to use.

Murine-specific IgE - 1 vial of IgE Standard in a protein buffer, 5 ng/ml

Conjugate Concentrate - 1 vial of a 100-fold concentrated biotinylated anti-IgE antibody in a stabilizing buffer

HRP Streptavidin Concentrate - 1 vial of a 100-fold concentrated streptavidin-HRP

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
- Measuring cylinders
- Distilled or deionized water
- Squirt bottle or automated microplate washer
- Microplate reader capable of measuring at 450 nm

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.

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 manually, 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.

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 before use. All diluents required are provided in the Ancillary Reagent Kit Catalog number M036080.

Wash Buffer (1X) in Ancillary Kit M036080 - 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 100 µL of Conjugate Concentrate into 9.9 mL of Conjugate diluent. Discard after use.

Streptavidin-HRP (1X) - **Prepare within 30 minutes of use and keep protected from light**. Add 100 µL Streptavidin-HRP Concentrate to 9.9 mL of HRP Diluent. Prepare fresh Streptavidin-HRP (1X) for each assay. If running less than a full plate, prepare only the amount needed.

Standards -

Murine IgE Serial Standard Dilution:

- Label 8 standard tubes. One of the standard tubes will contain the Assay Diluent as the zero standard.
- The supplied standard has a concentration of 5ng/ml of murine IgE. Create a 1:5 dilution by adding 100µl of the standard into 400 µl of Assay Diluent to create an initial diluted concentration of 1000 pg/ml.
- Add 250 µL Assay Diluent into the remaining tubes. Perform a 6-step serial dilution 1:2 starting with the 1000 pg/ml standard. Use the diluent as the 0 pg/ml standard.
- The 1000 pg/ml standard serves as the high standard and Assay Diluent serves as the zero (0 pg/mL) standard. The IgE standards will range from 15.6 pg/ml to 1000 pg/ml.

Recommended Sample Dilution:

- Samples are recommended to be diluted at least 1:10

ASSAY PROTOCOL

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 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. Add 100 µL of Standard or diluted sample in duplicate to each well. Cover with the plate sealer provided and incubate for 1 hour at 20-25 °C.
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 Conjugate (1X) to each well. Cover with the plate sealer provided and incubate for 1 hour at 20-25 °C.
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 for 30 minutes at 20-25 °C. **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 10 minutes at 20-25 °C. **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 to each well.
Cover with plate sealer and incubate 1 hr. at 20-25 °C.

Aspirate and wash 4 times.

Add 100 µL of Conjugate (1X) to each well.
Cover with plate sealer and incubate 1 hr. at 20-25 °C.

Aspirate and wash 4 times.

Add 100 µL of diluted Streptavidin-HRP to each well. Incubate 30 min. at 20-25 °C. Protect from Light.

Aspirate and wash 4 times.

Add 100 µL of Substrate to each well. Incubate 10 min. at 20-25 °C. Protect from Light.

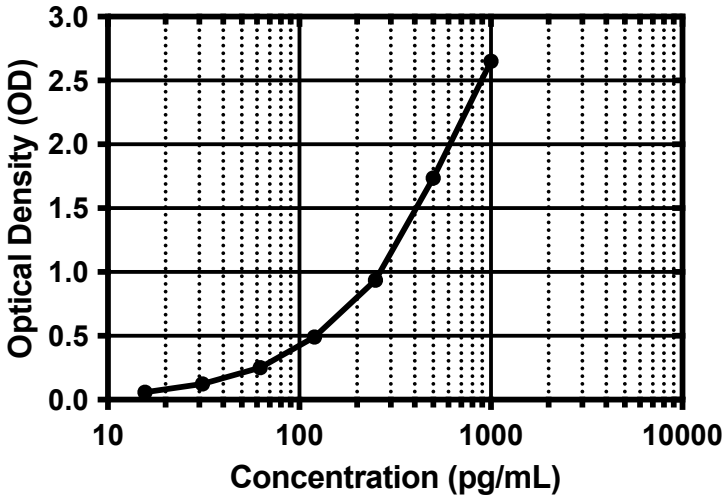
Add 100 µL of Stop Solution to each well. Read at 450 nm.

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 4-parameter logistic (4-PL) curve fit.

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

Range: 15.6-1000 pg/ml



PERFORMANCE CHARACTERISTICS

Sensitivity

The sensitivity of the assay is < 5.0 pg/ml

Reproducibility

Intra-assay Precision (Precision within an assay) - The intra-assay precision was measured by assaying three control samples 15-20 times on one plate.

Inter-assay Precision (Precision between assays) - The inter-assay precision was assessed by repeated measurements of three control samples in 15 successive assays with multiple users.

	Intra-assay Precision			Inter-assay Precision		
Control	1	2	3	1	2	3
Mean (ng/mL	86.2	151	379.1	81.3	157	356.3
Standard Deviation	2.55	4.79	15.3	5.88	12.45	16.28
CV (%)	3	3.2	4	7.2	7.9	4.6

CROSS-REACTIVITY

MonELISA® Murine IgE Assay is species specific and do not cross-react with other species.

LINEARITY

Four mouse sera samples were diluted in MD-1 Assay Diluent. Results in pg/mL are shown below:

Sample	Dilution	Expected pg/mL	Observed pg/mL	%Observed/Expected
A	1:50	---	212.9	---
	1:100	106.5	116.2	109.1%
	1:200	53.2	61.4	115.4%
	1:400	26.6	35.9	134.9%
B	1:50	---	936.6	---
	1:100	468.3	449.1	95.9%
	1:200	234.2	207.2	88.5%
	1:400	117.1	111.8	95.5%
C	1:50	---	899.5	---
	1:100	449.7	460.7	102.4%
	1:200	224.9	229.3	102.0%
	1:400	112.4	129.5	115.2%
D	1:50	---	180.0	---
	1:100	90.0	99.1	110.1%
	1:200	45.0	51.4	114.2%
	1:400	22.5	20.6	91.4%

RECOVERY

Various amounts of Mouse IgE were added to four different diluted mouse samples to determine the recovery. The results are described in the following table:

Sample	Endogenous (pg/mL)	Total IgE Added (pg/mL)	Expected Value (pg/mL)	Measured Value (pg/mL)	Recovery (%)
A	256.6	50	306.6	306.2	100%
		25	281.6	280.0	99%
B	46.9	50	96.9	86.7	89%
		25	71.9	69.2	96%
C	162.1	50	212.1	202.0	95%
		25	187.1	177.4	95%
D	161.7	50	211.7	194.1	92%
		25	186.7	175.2	94%

TROUBLESHOOTING

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

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