Mouse Eotaxin ELISA Kit

Enzyme-linked Immunosorbent Assay for quantitative detection of mouse Eotaxin

Catalog Numbers BMS6008 and BMS6008TEN

Pub. No. MAN0016673 **Rev.** A.0 (30)

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

The Mouse Eotaxin ELISA Kit is an enzyme-linked immunosorbent assay for the quantitative detection of mouse Eotaxin.

Summary

Eotaxin, also known as CCL11, is a member of the CC chemokine family of inflammatory and immunoregulatory cytokines.

Constitutive Eotaxin mRNA expression has been detected in multiple tissues, most often it appears to be induced by inflammatory cytokines such as IL-1, TNF α and IFN γ in fibroblasts, endothelial and epithelial cells. Following allergen challenge Eotaxin mRNA has been shown to be rapidly up-regulated in airway epithelium an alveolar macrophages. Eotaxin expression has also been detected in smooth muscle cells, chondrocytes and eosinophils.

Mouse Eotaxin activity is mediated by the mouse CC chemokine receptor CCR3, which, unlike human CCR3, can also be activated by mouse MIP-1 α.

Among CC chemokine familiy members, Eotaxin is functionally and structurally mostly related to the MCP/Eotaxin proteins. Mouse Eotaxin cDNA encodes a 97 amino acid residue precursor protein that is cleaved to generate the 74 aa residue mature protein.

Mouse Eotaxin has been shown to be a potent chemoattractant for eosinophils during inflammation and allergic reactions and to be involved in the growth of myeloid cell progenitors and the differentiation of mast cells during embryonic development. A role in numerous eosinophil-associated gastrointestinals disorders as food allergy, parasitic infections, allergic colitis and inflammatory Bowel disease has been described.

For literature update refer to our website.

Principles of the test

An anti-mouse Eotaxin coating antibody is adsorbed onto microwells.

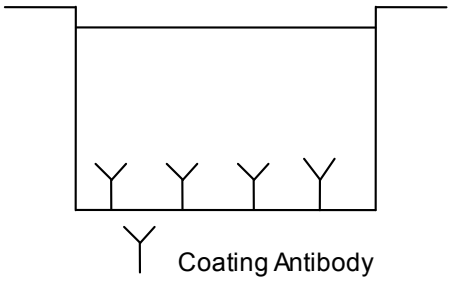

Fig. 1 Coated microwell

Mouse Eotaxin present in the sample or standard binds to antibodies adsorbed to the microwells. A biotin-conjugated anti-mouse Eotaxin antibody is added and binds to mouse Eotaxin captured by the first antibody.

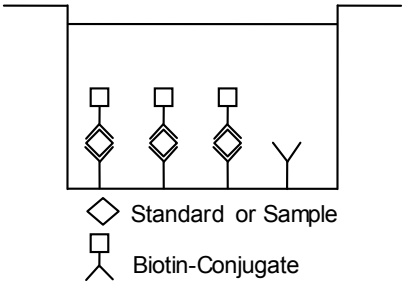

Fig. 2 First incubation

Following incubation unbound biotin-conjugated anti-mouse Eotaxin antibody is removed during a wash step. Streptavidin-HRP is added and binds to the biotin-conjugated anti-mouse Eotaxin antibody.

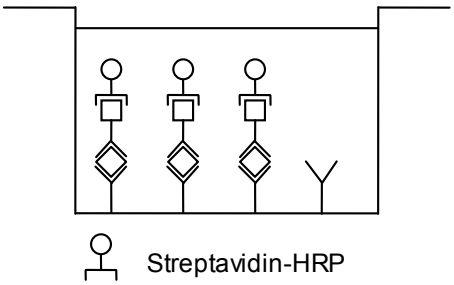

Fig. 3 Second incubation

Following incubation unbound Streptavidin-HRP is removed during a wash step, and substrate solution reactive with HRP is added to the wells.

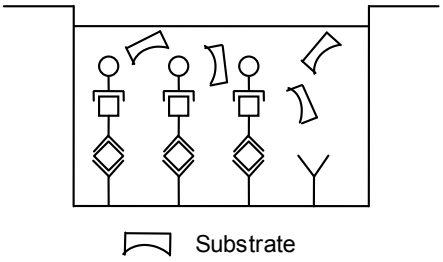

Fig. 4 Third incubation

A colored product is formed in proportion to the amount of mouse Eotaxin present in the sample or standard. The reaction is terminated by addition of acid and absorbance is measured at 450 nm. A standard curve is prepared from 7 mouse Eotaxin standard dilutions and mouse Eotaxin sample concentration determined.

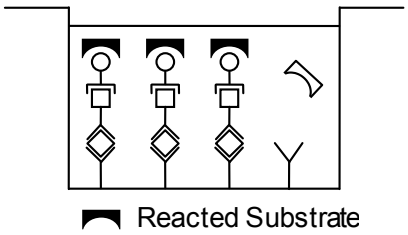

Fig. 5 Stop reaction

Reagents provided

Reagents for mouse Eotaxin ELISA BMS6008 (96 tests)

1 aluminum pouch with a Microwell Plate (12 strips of 8 wells each) coated with polyclonal antibody to mouse Eotaxin

1 vial (70 µL) Biotin-Conjugate anti-mouse Eotaxin polyclonal antibody

1 vial (150 µL) Streptavidin-HRP

2 vials mouse Eotaxin Standard lyophilized, 4 ng/mL upon reconstitution

1 vial (12 mL) Sample Diluent

1 vial (5 mL) Assay Buffer Concentrate 20x (PBS with 1% Tween 20, 10% BSA)

1 bottle (50 mL) Wash Buffer Concentrate 20x (PBS with 1% Tween 20)

1 vial (15 mL) Substrate Solution (tetramethyl-benzidine)

1 vial (15 mL) Stop Solution (1M Phosphoric acid)

4 Adhesive Films

Reagents for mouse Eotaxin ELISA BMS6008TEN (10x96 tests)

10 aluminum pouches with a Microwell Plate (12 strips of 8 wells each) coated with polyclonal antibody to mouse Eotaxin

10 vials (70 µL) Biotin-Conjugate anti-mouse Eotaxin polyclonal antibody

10 vials (150 µL) Streptavidin-HRP

10 vials mouse Eotaxin Standard lyophilized, 4 ng/mL upon reconstitution

7 vials (12 mL) Sample Diluent

2 vials (5 mL) Assay Buffer Concentrate 20x (PBS with 1% Tween 20, 10% BSA and 8% Red Dye)

4 bottles (50 mL) Wash Buffer Concentrate 20x (PBS with 1% Tween 20)

10 vials (15 mL) Substrate Solution (tetramethyl-benzidine)

1 vial (100 mL) Stop Solution (1M Phosphoric acid)

20 Adhesive Films

Storage instructions – ELISA kit

Store kit reagents between 2°C and 8°C. Immediately after use remaining reagents should be returned to cold storage (2°C to 8°C). Expiry of the kit and reagents is stated on labels.

Expiry of the kit components can only be guaranteed if the components are stored properly, and if, in case of repeated use of one component, this reagent is not contaminated by the first handling.

Sample collection and storage instructions

Cell culture supernatant and serum were tested with this assay. Other biological samples might be suitable for use in the assay. Remove serum from the clot as soon as possible after clotting. Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic samples.

Samples should be aliquoted and must be stored frozen at –20°C to avoid loss of bioactive mouse Eotaxin. If samples are to be run within 24 hours, they may be stored at 2°C to 8°C (refer to "Sample stability" on page 6). Avoid repeated freeze-thaw cycles. Prior to assay, the frozen sample should be brought to room temperature slowly and mixed gently.

Materials required but not provided

- 5 mL and 10 mL graduated pipettes
- 5 µL to 1000 µL adjustable single channel micropipettes with disposable tips
- 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
- Multichannel micropipette reservoir
- Beakers, flasks, cylinders necessary for preparation of reagents
- Device for delivery of wash solution (multichannel wash bottle or automatic wash system)
- Microwell strip reader capable of reading at 450 nm (620 nm as optional reference wave length)
- Glass-distilled or deionized water
- Statistical calculator with program to perform regression analysis

Precautions for use

- All chemicals should be considered as 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. Wear suitable protective clothing such as laboratory overalls, safety glasses, and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water. See material safety data sheet(s) and/or safety statement(s) for specific advice.
- Reagents are intended for research use only and are not for use in diagnostic or therapeutic procedures.
- Do not mix or substitute reagents with those from other lots or other sources.
- Do not use kit reagents beyond expiration date on label.
- Do not expose kit reagents to strong light during storage or incubation.
- Do not pipet by mouth.
- Do not eat or smoke in areas where kit reagents or samples are handled.
- Avoid contact of skin or mucous membranes with kit reagents or samples.
- Rubber or disposable latex gloves should be worn while handling kit reagents or samples.
- Avoid contact of substrate solution with oxidizing agents and metal.
- Avoid splashing or generation of aerosols.
- To avoid microbial contamination or cross-contamination of reagents or samples that may invalidate the test, use disposable pipette tips and/or pipettes.
- Use clean, dedicated reagent trays for dispensing the conjugate and substrate reagent.
- Exposure to acid inactivates the conjugate.
- Glass-distilled water or deionized water must be used for reagent preparation.
- Substrate solution must be at room temperature prior to use.
- Decontaminate and dispose samples and all potentially contaminated materials as if they could contain infectious agents. The preferred method of decontamination is autoclaving for a minimum of 1 hour at 121.5°C.
- Liquid wastes not containing acid and neutralized waste may be mixed with sodium hypochlorite in volumes such that the final mixture contains 1.0% sodium hypochlorite. Allow 30 minutes for effective decontamination. Liquid waste containing acid must be neutralized prior to the addition of sodium hypochlorite.

Preparation of reagents

1. Buffer concentrates should be brought to room temperature and should be diluted before starting the test procedure.
2. If crystals have formed in the Buffer Concentrates, warm them gently until they have completely dissolved.

Wash buffer (1x)

1. Pour entire contents (50 mL) of the Wash Buffer Concentrate (20x) into a clean 1000 mL graduated cylinder. Bring to final volume of 1000 mL with glass-distilled or deionized water.
2. Mix gently to avoid foaming.
3. Transfer to a clean wash bottle and store at 2°C to 25°C. Please note that Wash Buffer (1x) is stable for 30 days.
4. Wash Buffer (1x) may also be prepared as needed according to the following table:

Number of Strips	Wash Buffer Concentrate (20x) (mL)	Distilled Water (mL)
1 - 6	25	475
1 - 12	50	950

Assay buffer (1x)

1. Pour the entire contents (5 mL) of the Assay Buffer Concentrate (20x) into a clean 100 mL graduated cylinder. Bring to final volume of 100 mL with distilled water. Mix gently to avoid foaming.
2. Store at 2°C to 8°C. Please note that the Assay Buffer (1x) is stable for 30 days.
3. Assay Buffer (1x) may also be prepared as needed according to the following table:

Number of Strips	Assay Buffer Concentrate (20x) (mL)	Distilled Water (mL)
1 - 6	2.5	47.5
1 - 12	5.0	95.0

Biotin-Conjugate

Note: The Biotin-Conjugate should be used within 30 minutes after dilution.

Make a 1:100 dilution of the concentrated Biotin-Conjugate solution with Assay Buffer (1x) in a clean plastic tube as needed according to the following table:

Number of Strips	Biotin-Conjugate (mL)	Assay Buffer (1x) (mL)
1 - 6	0.03	2.97
1 - 12	0.06	5.94

Streptavidin-HRP

Note: The Streptavidin-HRP should be used within 30 minutes after dilution.

Make a 1:100 dilution of the concentrated Streptavidin-HRP solution with Assay Buffer (1x) in a clean plastic tube as needed according to the following table:

Number of Strips	Streptavidin-HRP (mL)	Assay Buffer (1x) (mL)
1 - 6	0.06	5.94
1 - 12	0.12	11.88

Mouse Eotaxin standard

1. Reconstitute mouse Eotaxin standard by addition of distilled water. Reconstitution volume is stated on the label of the standard vial. Allow the reconstituted standard to sit for 10-30 minutes.
2. Swirl or mix gently to insure complete and homogeneous solubilization (concentration of reconstituted standard = 4000 pg/mL).

The standard has to be used immediately after reconstitution and cannot be stored.

External standard dilution

1. Label 7 tubes, one for each standard point: S1, S2, S3, S4, S5, S6, S7.
2. Then prepare 1:2 serial dilutions for the standard curve as follows: Pipette 225 µL of Sample Diluent into each tube.
3. Pipette 225 µL of reconstituted standard (concentration = 4000 pg/mL) into the first tube, labeled S1, and mix (concentration of standard 1 = 2000 pg/mL).
4. Pipette 225 µL of this dilution into the second tube, labeled S2, and mix thoroughly before the next transfer.
5. Repeat serial dilutions 5 more times thus creating the points of the standard curve (see Figure 6).

   Sample Diluent serves as blank.

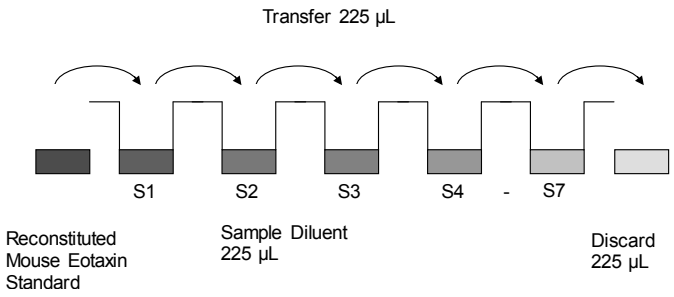

Fig. 6 Dilute standards - tubes

6.

Test protocol

1. Determine the number of microwell strips required to test the desired number of samples plus appropriate number of wells needed for running blanks and standards. Each sample, standard, blank and optional control sample should be assayed in duplicate. Remove extra microwell strips from holder and store in foil bag with the desiccant provided at 2°C to 8°C sealed tightly.
2. Wash the microwell strips twice with approximately 400 µL Wash Buffer per well with thorough aspiration of microwell contents between washes. Allow the Wash Buffer to sit in the wells for about 10 – 15 seconds before aspiration. Take care not to scratch the surface of the microwells.

   After the last wash step, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffer. Use the microwell strips immediately after washing. Alternatively microwell strips can be placed upside down on a wet absorbent paper for not longer than 15 minutes. Do not allow wells to dry.
3. Standard dilution on the microwell plate (Alternatively the standard dilution can be prepared in tubes - see “External standard dilution“ on page 3):

   Add 100 µL of Sample Diluent in duplicate to all standard wells. Pipette 100 µL of prepared standard (see “Mouse Eotaxin standard“ on page 3, concentration = 4000 pg/mL) in duplicate into well A1 and A2 (see Table 1). Mix the contents of wells A1 and A2 by repeated aspiration and ejection (concentration of standard 1, S1 = 2000 pg/mL), and transfer 100 µL to wells B1 and B2, respectively (see Figure 7). Take care not to scratch the inner surface of the microwells. Continue this procedure 5 times, creating two rows of mouse Eotaxin standard dilutions ranging from 2000.0 to 31.3 pg/mL. Discard 100 µL of the contents from the last microwells (G1, G2) used.

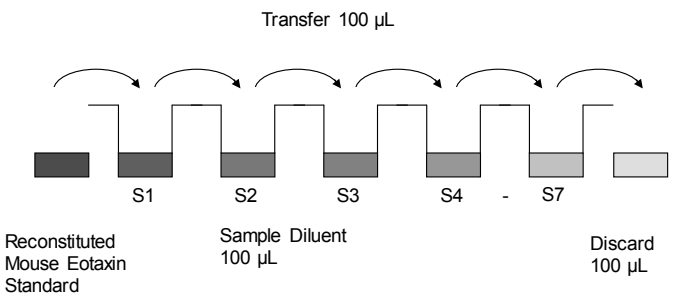

Fig. 7 Dilute standards - microwell plate

Table 1 Example of the arrangement of blanks, standards and samples in the microwell strips.

	1	2	3	4
A	Standard 1 2000.0 pg/mL	Standard 1 2000.0 pg/mL	Sample 1	Sample 1
B	Standard 2 1000.0 pg/mL	Standard 2 1000.0 pg/mL	Sample 2	Sample 2
C	Standard 3 500.0 pg/mL	Standard 3 500.0 pg/mL	Sample 3	Sample 3
D	Standard 4 250.0 pg/mL	Standard 4 250.0 pg/mL	Sample 4	Sample 4
E	Standard 5 125.0 pg/mL	Standard 5 125.0 pg/mL	Sample 5	Sample 5
F	Standard 6 62.5 pg/mL	Standard 6 62.5 pg/mL	Sample 6	Sample 6
G	Standard 7 31.3 pg/mL	Standard 7 31.3 pg/mL	Sample 7	Sample 7
H	Blank	Blank	Sample 8	Sample 8

In case of an external standard dilution (see "External standard dilution" on page 3), pipette 100 µL of these standard dilutions (S1 - S7) in the standard wells according to Table 1.

4. Add 100 µL of Sample Diluent in duplicate to the blank wells.
5. Add 50 µL of Sample Diluent to the sample wells.
6. Add 50 µL of each sample in duplicate to the sample wells.
7. Prepare Biotin-Conjugate (see "Biotin-Conjugate" on page 3).
8. Add 50 µL of Biotin-Conjugate to all wells.
9. Cover with an adhesive film and incubate at room temperature (18°C to 25°C) for 2 hours on a microplate shaker.
10. Prepare Streptavidin-HRP (refer to "Streptavidin-HRP" on page 3).
11. Remove adhesive film and empty wells. Wash microwell strips 4 times according to point 2. of the test protocol. Proceed immediately to the next step.
12. Add 100 µL of diluted Streptavidin-HRP to all wells, including the blank wells.
13. Cover with an adhesive film and incubate at room temperature (18°C to 25°C) for 1 hour on a microplate shaker.
14. Remove adhesive film and empty wells. Wash microwell strips 4 times according to point 2. of the test protocol. Proceed immediately to the next step.
15. Pipette 100 µL of TMB Substrate Solution to all wells.
16. Incubate the microwell strips at room temperature (18°C to 25°C) for about 10 minutes. Avoid direct exposure to intense light.

    The color development on the plate should be monitored and the substrate reaction stopped (see next point of this protocol) before positive wells are no longer properly recordable. Determination of the ideal time period for color development has to be done individually for each assay.

    It is recommended to add the stop solution when the highest standard has developed a dark blue color. Alternatively the color development can be monitored by the ELISA reader at 620 nm. The substrate reaction should be stopped as soon as Standard 1 has reached an OD of 0.9 – 0.95.

17. Stop the enzyme reaction by quickly pipetting 100 µL of Stop Solution into each well. It is important that the Stop Solution is spread quickly and uniformly throughout the microwells to completely inactivate the enzyme. Results must be read immediately after the Stop Solution is added or within one hour if the microwell strips are stored at 2°C to 8°C in the dark.
18. Read absorbance of each microwell on a spectro-photometer using 450 nm as the primary wave length (optionally 620 nm as the reference wave length; 610 nm to 650 nm is acceptable). Blank the plate reader according to the manufacturer's instructions by using the blank wells. Determine the absorbance of both the samples and the standards.

Note: In case of incubation without shaking the obtained O.D. values may be lower than indicated below. Nevertheless the results are still valid.

Calculation of results

- Calculate the average absorbance values for each set of duplicate standards and samples. Duplicates should be within 20% of the mean value.
- Create a standard curve by plotting the mean absorbance for each standard concentration on the ordinate against the mouse Eotaxin concentration on the abscissa. Draw a best fit curve through the points of the graph (a 5-parameter curve fit is recommended).
- To determine the concentration of circulating mouse Eotaxin for each sample, first find the mean absorbance value on the ordinate and extend a horizontal line to the standard curve. At the point of intersection, extend a vertical line to the abscissa and read the corresponding mouse Eotaxin concentration.
- If instructions in this protocol have been followed, samples have been diluted 1:2 (50 µL sample + 50 µL Sample Diluent) and the concentration read from the standard curve must be multiplied by the dilution factor (x 2).
- Calculation of samples with a concentration exceeding standard 1 may result in incorrect, low mouse Eotaxin levels. Such samples require further external predilution according to expected mouse Eotaxin values with Sample Diluent in order to precisely quantitate the actual mouse Eotaxin level.
- It is suggested that each testing facility establishes a control sample of known mouse Eotaxin concentration and runs this additional control with each assay. If the values obtained are not within the expected range of the control, the assay results may be invalid.
- A representative standard curve is shown in Figure 8. This curve cannot be used to derive test results. Each laboratory must prepare a standard curve for each group of microwell strips assayed.

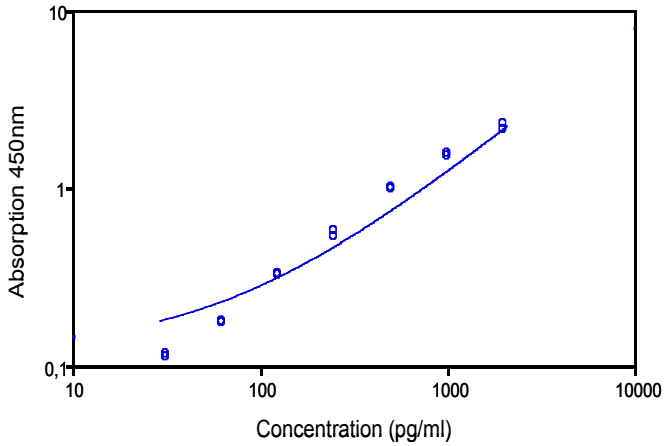

Fig. 8 Representative standard curve for mouse Eotaxin ELISA. Mouse Eotaxin was diluted in serial 2-fold steps in Sample Diluent. Do not use this standard curve to derive test results. A standard curve must be run for each group of microwell strips assayed.

Table 2 Typical data using the mouse Eotaxin ELISA

Measuring wavelength: 450 nm

Reference wavelength: 620 nm

Standard	Mouse Eotaxin Concentration (pg/mL)	O.D. at 450 nm	Mean O.D. at 450 nm	C.V. (%)
1	2000.0	2.306 2.149	2.228	5.0
2	1000.0	1.509 1.577	1.543	3.1
3	500.0	1.001 0.981	0.991	1.4
4	250.0	0.534 0.573	0.554	5.0
5	125.0	0.323 0.329	0.326	1.3
6	62.5	0.176 0.178	0.177	1.0
7	31.3	0.112 0.117	0.115	3.2
Blank	0.0	0.041 0.036	0.039	9.2

The OD values of the standard curve may vary according to the conditions of assay performance (e.g. operator, pipetting technique, washing technique or temperature effects). Furthermore shelf life of the kit may affect enzymatic activity and thus color intensity. Values measured are still valid.

Limitations

- Since exact conditions may vary from assay to assay, a standard curve must be established for every run.
- Bacterial or fungal contamination of either screen samples or reagents or cross-contamination between reagents may cause erroneous results.
- Disposable pipette tips, flasks or glassware are preferred, reusable glassware must be washed and thoroughly rinsed of all detergents before use.
- Improper or insufficient washing at any stage of the procedure will result in either false positive or false negative results. Empty wells completely before dispensing fresh wash solution, fill with Wash Buffer as indicated for each wash cycle and do not allow wells to sit uncovered or dry for extended periods.

Performance characteristics

Sensitivity

The limit of detection of mouse Eotaxin defined as the analyte concentration resulting in an absorbance significantly higher than that of the dilution medium (mean plus 2 standard deviations) was determined to be 3.6 pg/mL (mean of 6 independent assays).

Reproducibility

Intra-assay

Reproducibility within the assay was evaluated in 3 independent experiments. Each assay was carried out with 6 replicates of 8 serum samples containing different concentrations of mouse Eotaxin. Two standard curves were run on each plate. Data below show the mean mouse Eotaxin concentration and the coefficient of variation for each sample (see Table 3). The calculated overall intra-assay coefficient of variation was 5.6%.

Table 3 The mean mouse Eotaxin concentration and the coefficient of variation for each sample

Sample	Experiment	Mean Mouse Eotaxin Concentration (pg/mL)	Coefficient of Variation (%)
1	1 2 3	749.6 761.4 812.6	9.0 6.0 7.0
2	1 2 3	326.1 293.3 335.1	3.0 4.0 5.0
3	1 2 3	160.3 156.1 156.6	5.0 4.0 4.0
4	1 2 3	136.7 118.3 130.3	4.0 6.0 7.0
5	1 2 3	950.3 685.9 839.9	10.0 3.0 7.0
6	1 2 3	312.9 260.0 330.1	10.0 4.0 5.0
7	1 2 3	160.1 137.1 170.6	6.0 6.0 4.0
8	1 2 3	155.7 112.4 138.1	5.0 7.0 6.0

Inter-assay

Assay to assay reproducibility within one laboratory was evaluated in 3 independent experiments. Each assay was carried out with 6 replicates of 8 serum samples containing different concentrations of mouse Eotaxin. Two standard curves were run on each plate. Data below show the mean mouse Eotaxin concentration and the coefficient of variation calculated on 18 determinations of each sample (see Table 4). The calculated overall inter-assay coefficient of variation was 9.4%.

Table 4 The mean mouse Eotaxin concentration and the coefficient of variation of each sample

Sample	Mean Mouse Eotaxin Concentration (pg/mL)	Coefficient of Variation (%)
1	774.5	4.3
2	318.2	6.9
3	157.7	1.4
4	128.5	7.3
5	825.4	16.1
6	301.0	12.1
7	156.0	11.0
8	135.4	16.1

Spike recovery

The spike recovery was evaluated by spiking 4 levels of mouse Eotaxin into serum. Recoveries were determined in 3 independent experiments with 6 replicates each.

The unspiked serum was used as blank in these experiments.

The overall mean recovery was 66%.

Dilution parallelism

Serum samples with different levels of mouse Eotaxin were analyzed at serial 2-fold dilutions with 4 replicates each.

The recovery ranged from 103% to 121% with an overall recovery of 114%.

Sample	Dilution	Expected Mouse Eotaxin Concentration (pg/mL)	Observed Mouse Eotaxin Concentration (pg/mL)	Recovery of Expected Mouse Eotaxin Concentration (%)
1	1:2	-	1035.5	-
	1:4	517.8	606.8	117.2
	1:8	303.4	363.0	119.6
	1:16	181.5	218.7	120.5
2	1:2	-	326.1	-
	1:4	163.1	190.0	116.5
	1:8	95.0	100.8	106.1
	1:16	50.4	53.6	106.5
3	1:2	-	1074.2	-
	1:4	537.1	638.6	118.9
	1:8	319.3	374.3	117.2
	1:16	187.1	209.3	111.9
4	1:2	-	361.9	-
	1:4	181.0	198.1	109.5
	1:8	99.1	114.8	115.9
	1:16	57.4	59.1	103.0

Sample stability

Freeze-Thaw stability

Aliquots of serum samples were stored at -20°C and thawed 5 times, and the mouse Eotaxin levels determined. There was no significant loss of mouse Eotaxin immunoreactivity detected by freezing and thawing.

Storage stability

Aliquots of serum samples were stored at -20°C, 2°C to 8°C, room temperature, and at 37°C, and the mouse Eotaxin level determined after 24 h. There was no significant loss of mouse Eotaxin immunoreactivity detected during storage under above conditions.

Specificity

The interference of circulating factors of the immune system was evaluated by spiking these proteins at physiologically relevant concentrations into a mouse Eotaxin positive serum.

There was no crossreactivity detected.

Expected values

A panel of 6 serum pools from randomly selected apparently healthy mice was tested for mouse Eotaxin. The detected mouse Eotaxin levels ranged between 98 and 236 pg/mL with a mean level of 176 pg/mL. The levels measured may vary with the sample collection used.

Reagent preparation summary

Wash buffer (1x)

Add Wash Buffer Concentrate 20x (50 mL) to 950 mL distilled water.

Number of Strips	Wash Buffer Concentrate (mL)	Distilled Water (mL)
1 - 6	25	475
1 - 12	50	950

Assay buffer (1x)

Add Assay Buffer Concentrate 20x (5 mL) to 95 mL distilled water.

Number of Strips	Assay Buffer Concentrate (mL)	Distilled Water (mL)
1 - 6	2.5	47.5
1 - 12	5.0	95.0

Biotin-Conjugate

Make a 1:100 dilution of Biotin-Conjugate in Assay Buffer (1x):

Number of Strips	Biotin-Conjugate (mL)	Assay Buffer (1x) (mL)
1 - 6	0.03	2.97
1 - 12	0.06	5.94

Streptavidin-HRP

Make a 1:100 dilution of Streptavidin-HRP in Assay Buffer (1x):

Number of Strips	Streptavidin-HRP (mL)	Assay Buffer (1x) (mL)
1 - 6	0.06	5.94
1 - 12	0.12	11.88

Mouse Eotaxin standard

Reconstitute lyophilized mouse Eotaxin standard with distilled water. (Reconstitution volume is stated on the label of the standard vial.)

Test protocol summary

1. Determine the number of microwell strips required.
2. Wash microwell strips twice with Wash Buffer.
3. Standard dilution on the microwell plate: Add 100 µL Sample Diluent, in duplicate, to all standard wells. Pipette 100 µL prepared standard into the first wells and create standard dilutions by transferring 100 µL from well to well. Discard 100 µL from the last wells.

   Alternatively external standard dilution in tubes (see "External standard dilution" on page 3): Pipette 100 µL of these standard dilutions in the microwell strips.
4. Add 100 µL Sample Diluent, in duplicate, to the blank wells.
5. Add 50 µL Sample Diluent to sample wells.
6. Add 50 µL sample in duplicate, to designated sample wells.
7. Prepare Biotin-Conjugate.
8. Add 50 µL Biotin-Conjugate to all wells.
9. Cover microwell strips and incubate 2 hours at room temperature (18°C to 25°C).
10. Prepare Streptavidin-HRP.
11. Empty and wash microwell strips 4 times with Wash Buffer.
12. Add 100 µL diluted Streptavidin-HRP to all wells.
13. Cover microwell strips and incubate 1 hour at room temperature (18°C to 25°C).
14. Empty and wash microwell strips 4 times with Wash Buffer.
15. Add 100 µL of TMB Substrate Solution to all wells.
16. Incubate the microwell strips for about 10 minutes at room temperature (18°C to 25°C).
17. Add 100 µL Stop Solution to all wells.
18. Blank microwell reader and measure color intensity at 450 nm.

Note: If instructions in this protocol have been followed, samples have been diluted 1:2 (50 µL sample + 50 µL Sample Diluent) and the concentration read from the standard curve must be multiplied by the dilution factor (x 2).

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