ab206985 – Eotaxin-2 (CCL24) Mouse SimpleStep ELISA® Kit

Instructions for Use

For the quantitative measurement of Eotaxin-2 (CCL24) mouse protein in mouse serum, plasma, and cell culture supernatants.

This product is for research use only and is not intended for diagnostic use.

Version 4a Last Updated 18 March 2024

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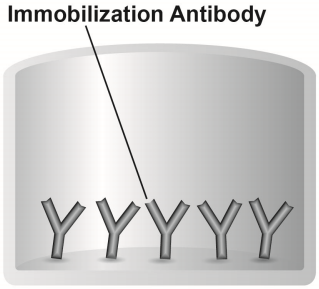
1. BACKGROUND

Abcam's Eotaxin-2 (CCL24) *in vitro* SimpleStep ELISA® (Enzyme-Linked Immunosorbent Assay) kit is designed for the quantitative measurement of Eotaxin-2 in mouse serum, plasma, and cell culture supernatants.

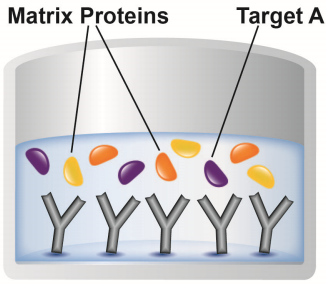
The SimpleStep ELISA® employs an affinity tag labeled capture antibody and a reporter conjugated detector antibody which immunocapture the sample analyte in solution. This entire complex (capture antibody/analyte/detector antibody) is in turn immobilized via immunoaffinity of an anti-tag antibody coating the well. To perform the assay, samples or standards are added to the wells, followed by the antibody mix. After incubation, the wells are washed to remove unbound material. TMB substrate is added and during incubation is catalyzed by HRP, generating blue coloration. This reaction is then stopped by addition of Stop Solution completing any color change from blue to yellow. Signal is generated proportionally to the amount of bound analyte and the intensity is measured at 450 nm. Optionally, instead of the endpoint reading, development of TMB can be recorded kinetically at 600 nm.

Eotaxin-2, also known as CCL24, MPIF-2 or Ckβ-6, is a C-C chemokine originally identified in activated monocytes. Eotaxin-2 is expressed in activated T lymphocytes, GM-CSF treated macrophages and dermal fibroblasts. Mouse eotaxin-2 is a highly glycosylated protein which requires maturation by removal of a 26 aminoacid signal peptide prior to secretion. Secretion of Eotaxin-2 is known to recruit and activate CCR3-bearing cells particularly eosinophils as well as basophils. Activation of eosinophils generates reactive oxygen species, lipid mediators of inflammation and degranulation of toxic granule proteins. Chemotaxis and activation of basophils, previously primed with IL-3, lead to release of histamine and leukotriene C4.

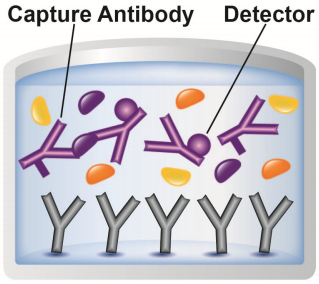
2. ASSAY SUMMARY



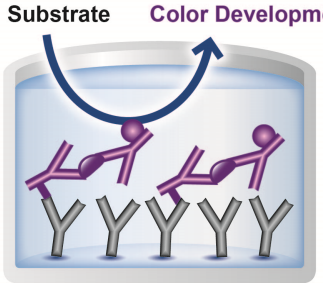
Remove appropriate number of antibody coated well strips. Equilibrate all reagents to room temperature. Prepare all reagents, samples, and standards as instructed.



Add standard or sample to appropriate wells.



Add Antibody Cocktail to all wells. Incubate at room temperature.



Aspirate and wash each well. Add TMB Substrate to each well and incubate. Add Stop Solution at a defined endpoint. Alternatively, record color development kinetically after TMB substrate addition.

3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at 2-8ºC immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Reagent and Standard Preparation sections.

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)
Mouse Eotaxin-2 Capture Antibody (lyophilized)	1 vial	+2-8ºC
10X Mouse Eotaxin-2 Detector Antibody	600 µL	+2-8ºC
Mouse Eotaxin-2 Lyophilized Recombinant Protein	2 Vials	+2-8ºC
Antibody Diluent CPI2	6 mL	+2-8ºC
10X Wash Buffer PT	20 mL	+2-8ºC
TMB Substrate	12 mL	+2-8ºC
Stop Solution	12 mL	+2-8ºC
Sample Diluent NS	50 mL	+2-8ºC
Pre-Coated 96 Well Microplate (12 x 8 well strips)	96 Wells	+2-8ºC
Plate Seal	1	+2-8ºC

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Microplate reader capable of measuring absorbance at 450 or 600 nm.
- Method for determining protein concentration (BCA assay recommended).
- Deionized water.
- PBS (1.4 mM KH2PO4, 8 mM Na2HPO4, 140 mM NaCl, 2.7 mM KCl, pH 7.4).
- Multi- and single-channel pipettes.
- Tubes for standard dilution.
- Plate shaker for all incubation steps.
- Phenylmethylsulfonyl Fluoride (PMSF) (or other protease inhibitors).

7. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

8. TECHNICAL HINTS

- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Complete removal of all solutions and buffers during wash steps is necessary to minimize background.
- As a guide, typical ranges of sample concentration for commonly used sample types are shown below in Sample Preparation (section 11).
- All samples should be mixed thoroughly and gently.
- Avoid multiple freeze/thaw of samples.
- Incubate ELISA plates on a plate shaker during all incubation steps.
- When generating positive control samples, it is advisable to change pipette tips after each step.
- **To avoid high background always add samples or standards to the well before the addition of the antibody cocktail.**
- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**

9. REAGENT PREPARATION

- Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells. **The sample volumes below are sufficient for 48 wells (6 x 8-well strips); adjust volumes as needed for the number of strips in your experiment.**
- Prepare only as much reagent as is needed on the day of the experiment. Capture and Detector Antibodies have only been tested for stability in the provided 10X formulations.

9.1 1X Wash Buffer PT

Prepare 1X Wash Buffer PT by diluting 10X Wash Buffer PT with deionized water. To make 50 mL 1X Wash Buffer PT combine 5 mL 10X Wash Buffer PT with 45 mL deionized water. Mix thoroughly and gently.

9.2 10X Capture antibody

To reconstitute the lyophilized capture antibody, centrifuge the lyophilized capture at 10,000 g for 2 minutes. Add 660 µL of Sample Diluent NS, let sit at room temperature for 10 minutes and resuspend well by inverting the tube by hand and gently pipetting. Unused antibody can be stored frozen at -20ºC. Avoid repeated freeze-thaw cycles.

9.3 Antibody Cocktail

Prepare Antibody Cocktail by diluting the capture and detector antibodies in Antibody Diluent CPI2. To make 3 mL of the Antibody Cocktail combine 300 µL 10X Capture Antibody and 300 µL 10X Detector Antibody with 2.4 mL Antibody Diluent CPI2. Mix thoroughly and gently.

10. STANDARD PREPARATION

Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of positive controls for every use.

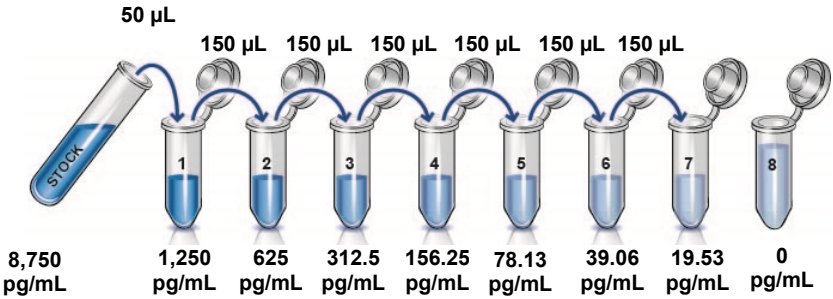
The following section describes the preparation of a standard curve for duplicate measurements (recommended).

10.1 **IMPORTANT:** If the protein standard vial has a volume identified on the label, reconstitute the Eotaxin-2 standard by adding that volume of Sample Diluent NS indicated on the label. Alternatively, if the vial has a mass identified, reconstitute the Eotaxin-2 standard by adding 1.25 mL Sample Diluent NS. Hold at room temperature for 10 minutes and mix gently. This is the 8,750 pg/mL **Stock Standard** Solution.

10.2 Label eight tubes, Standards 1– 8.

10.3 Add 300 µL Sample Diluent NS into tube number 1 and 150 µL of Sample Diluent NS into numbers 2-8.

10.4 Use the Stock Standard to prepare the following dilution series. Standard #8 contains no protein and is the Blank control:



11. SAMPLE PREPARATION

TYPICAL SAMPLE DYNAMIC RANGE	
Sample Type	**Range**
Mouse Serum	1:1 – 1:32
Mouse Plasma - Citrate	1:1 – 1:32
Mouse Plasma - EDTA	1:1 – 1:32
Mouse Lung Supernatants stimulated with human IL-4	1:20 – 1:640
Cell Culture Media	1:4 – 1:128

11.1 Plasma

Collect plasma using citrate or EDTA. Centrifuge samples at 2,000 x g for 10 minutes. Dilute samples into Sample Diluent NS and assay. Store un-diluted plasma samples at -20ºC or below for up to 3 months. Avoid repeated freeze-thaw cycles.

Note: Heparin plasma is not suitable for use with this kit.

11.2 Serum

Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2,000 x g for 10 minutes and collect serum. Dilute samples into Sample Diluent NS and assay. Store un-diluted serum at -20ºC or below. Avoid repeated freeze-thaw cycles.

11.3 Cell Culture Supernatants

Centrifuge cell culture media at 2,000 x g for 10 minutes to remove debris. Collect supernatants and dilute samples into Sample Diluent NS and assay. Store un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.

12. PLATE PREPARATION

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- Unused plate strips should be immediately returned to the foil pouch containing the desiccant pack, resealed and stored at 4°C.
- For each assay performed, a minimum of two wells must be used as the zero control.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Differences in well absorbance or “edge effects” have not been observed with this assay.

13. ASSAY PROCEDURE

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **It is recommended to assay all standards, controls and samples in duplicate.**

13.1 Prepare all reagents, working standards, and samples as directed in the previous sections.

13.2 Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4ºC storage.

13.3 Add 50 µL of all sample or standard to appropriate wells.

13.4 Add 50 µL of the Antibody Cocktail to each well.

13.5 Seal the plate and incubate for 1 hour at room temperature on a plate shaker set to 400 rpm.

13.6 Wash each well with 3 x 350 µL 1X Wash Buffer PT. Wash by aspirating or decanting from wells then dispensing 350 µL 1X Wash Buffer PT into each well. Complete removal of liquid at each step is essential for good performance. After the last wash invert the plate and blot it against clean paper towels to remove excess liquid.

13.7 Add 100 µL of TMB Substrate to each well and incubate for 10 minutes in the dark on a plate shaker set to 400 rpm.

Given variability in laboratory environmental conditions, optimal incubation time may vary between 5 and 20 minutes.

Note: The addition of Stop Solution will change the color from blue to yellow and enhance the signal intensity about 3X. To avoid signal saturation, proceed to the next step before the high concentration of the standard reaches a blue color of O.D.600 equal to 1.0.

13.8 Add 100 µL of Stop Solution to each well. Shake plate on a plate shaker for 1 minute to mix. Record the OD at 450 nm. This is an endpoint reading.

Alternative to 13.7 – 13.8: Instead of the endpoint reading at 450 nm, record the development of TMB Substrate kinetically. Immediately after addition of TMB Development Solution begin recording the blue color development with elapsed time in the microplate reader prepared with the following settings:

Mode:	Kinetic
Wavelength:	600 nm
Time:	up to 20 min
Interval:	20 sec - 1 min
Shaking:	Shake between readings

Note that an endpoint reading can also be recorded at the completion of the kinetic read by adding 100 µL Stop Solution to each well and recording the OD at 450 nm.

13.9 Analyze the data as described below.

14. CALCULATIONS

14.1 Calculate the average absorbance value for the blank control (zero) standards. Subtract the average blank control standard absorbance value from all other absorbance values.

14.2 Create a standard curve by plotting the average blank control subtracted absorbance value for each standard concentration (y-axis) against the target protein concentration (x-axis) of the standard. Use graphing software to draw the best smooth curve through these points to construct the standard curve.

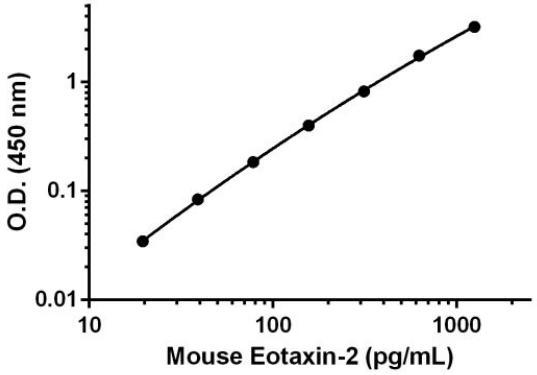
Note: Most microplate reader software or graphing software will plot these values and fit a curve to the data. A four parameter curve fit (4PL) is often the best choice; however, other algorithms (e.g. linear, semi-log, log/log, 4 parameter logistic) can also be tested to determine if it provides a better curve fit to the standard values.

14.3 Determine the concentration of the target protein in the sample by interpolating the blank control subtracted absorbance values against the standard curve. Multiply the resulting value by the appropriate sample dilution factor, if used, to obtain the concentration of target protein in the sample.

14.4 Samples generating absorbance values greater than that of the highest standard should be further diluted and reanalyzed. Similarly, samples which measure at an absorbance values less than that of the lowest standard should be retested in a less dilute form.

15. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed.



Standard Curve Measurements			
Conc. (pg/mL)	O.D. 450 nm		Mean O.D.
	1	2	
0	0.0709	0.0579	0.0644
19.53	0.1007	0.0972	0.0990
39.06	0.1518	0.1447	0.1483
78.13	0.2464	0.2505	0.2485
156.25	0.4663	0.4597	0.4630
312.5	0.8896	0.8788	0.8842
625	1.8129	1.8010	1.8070
1,250	3.2212	3.2954	3.2583

Figure 1. Example of the mouse Eotaxin-2 standard curve in Sample Diluent NS. The mouse Eotaxin-2 standard curve was prepared as described in Section 10. Raw data values are shown in the table. Background-subtracted data values (mean +/- SD) are graphed.

16. TYPICAL SAMPLE VALUES

SENSITIVITY –

The calculated minimal detectable dose (MDD) is 6.23 pg/mL. The MDD was determined by calculating the mean of zero standard replicates (n=24) and adding 2 standard deviations then extrapolating the corresponding concentration.

RECOVERY –

Three concentrations of mouse Eotaxin-2 were spiked in duplicate to the indicated biological matrix to evaluate signal recovery in the working range of the assay.

Sample Type	Average % Recovery	Range (%)
Mouse Serum (Neat)	100	98 - 101
Mouse Plasma – Citrate (Neat)	95	89 - 102
Mouse Plasma – EDTA (Neat)	98	94 - 105
Cell Culture Media (1:4)	94	90 - 99

LINEARITY OF DILUTION –

Linearity of dilution is determined based on interpolated values from the standard curve. Linearity of dilution defines a sample concentration interval in which interpolated target concentrations are directly proportional to sample dilution.

Native mouse Eotaxin-2 was measured in mouse lung supernatants stimulated with IL-4 in a 2-fold dilution series. Sample dilutions are made in Sample Diluent NS.

Recombinant mouse Eotaxin-2 was spiked into normal mouse serum, citrate and EDTA plasmas, and cell culture media and diluted in a 2-fold dilution series in Sample Diluent NS.

Dilution Factor	Interpolated value	Neat Mouse Serum	Neat Mouse Plasma (Citrate)	Neat Mouse Plasma (EDTA)	1:20 Mouse Lung Supernatants stimulated with IL-4	1:4 Cell Culture Media
1	pg/mL	587.79	587.07	559.45	1206.67	607.8
	% Expected value	**100**	**100**	**100**	**100**	**100**
2	pg/mL	342.78	324.64	307.68	605.31	289.62
	% Expected value	**117**	**111**	**110**	**100**	**95**
4	pg/mL	175.16	170.56	151.73	267.90	143.04
	% Expected value	**119**	**116**	**108**	**89**	**94**
8	pg/mL	86.98	82.48	71.21	123.06	72.98
	% Expected value	**118**	**112**	**102**	**82**	**96**
16	pg/mL	41.79	38.91	36.67	61.32	35.47
	% Expected value	**114**	**106**	**105**	**81**	**93**
32	pg/mL	20.24	18.33	17.77	31.10	19.02
	% Expected value	**110**	**100**	**102**	**82**	**100**

PRECISION –

Mean coefficient of variations of interpolated values from 3 concentrations of stimulated mouse lung supernatant within the working range of the assay.

	Intra-Assay	Inter-Assay
n=	8	3
CV (%)	3.42	4.66

DATA –

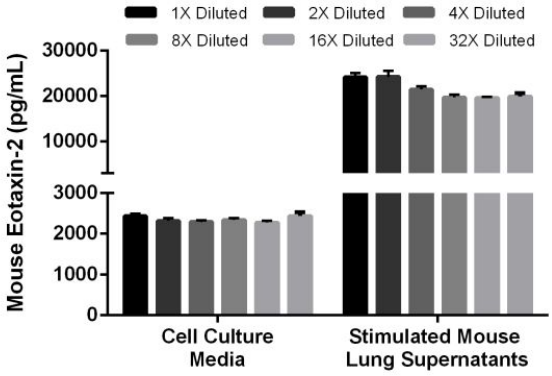


Figure 2. Linearity of dilution of mouse Eotaxin-2 in cell culture media and stimulated mouse lung supernatants. Recombinant mouse Eotaxin-2 was spiked into 1:4 cell culture media and diluted in a 2-fold dilution series in Sample Diluent NS. Native mouse Eotaxin-2 was measured in 1:20 stimulated mouse lung supernatants which was further diluted in a 2-fold dilution series in Sample Diluent NS. The concentrations of mouse Eotaxin-2 were measured in duplicate and interpolated from the mouse Eotaxin-2 standard curve and corrected for sample dilution. The interpolated dilution factor corrected values are graphed (mean +/- SD).

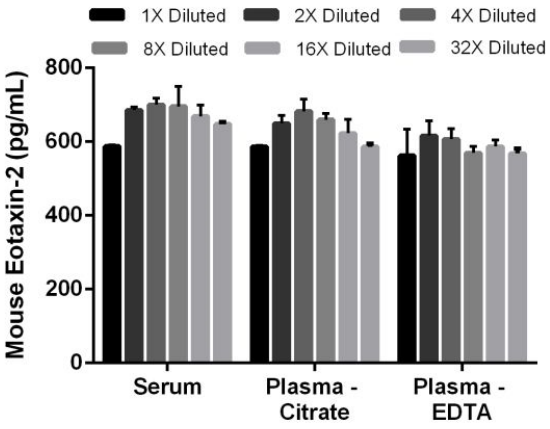


Figure 3. Spiked linearity of dilution of mouse Eotaxin-2 in normal serum and normal plasma samples. Recombinant mouse Eotaxin-2 was spiked into neat serum, neat citrate plasma, and neat EDTA plasma diluted in a 2-fold dilution series in Sample Diluent NS. The concentrations of mouse Eotaxin-2 were measured in duplicate and interpolated from the mouse Eotaxin-2 standard curve and corrected for sample dilution. The interpolated dilution factor corrected values are graphed (mean +/- SD).

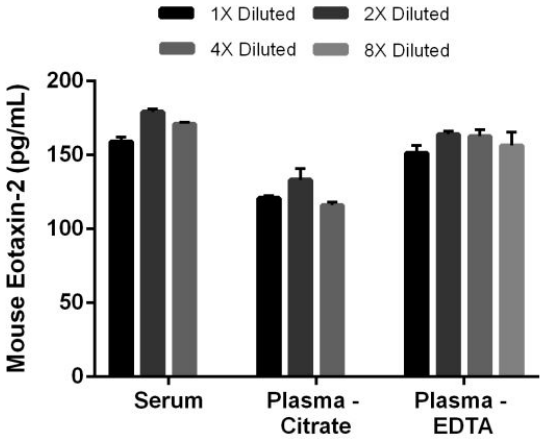


Figure 4. Native linearity of dilution of mouse Eotaxin-2 in normal serum and normal plasma samples. Native mouse Eotaxin-2 was measured in neat normal serum, neat normal citrate plasma, and neat normal EDTA plasma diluted in a 2-fold dilution series in Sample Diluent NS. The concentrations of mouse Eotaxin-2 were measured in duplicate and interpolated from the mouse Eotaxin-2 standard curve and corrected for sample dilution. The interpolated dilution factor corrected values are graphed (mean +/- SD). For reference, 8X or more diluted normal serum and normal citrate plasma samples interpolate below the standard curve. 16X or more diluted Normal EDTA plasma samples interpolate below the standard curve.

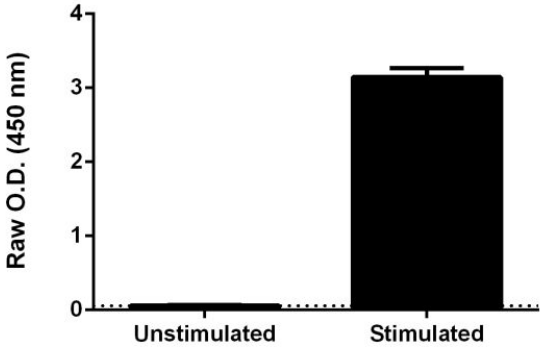


Figure 5. Assay specificity is demonstrated on mouse lung supernatants. Mouse lung supernatants were cultured in cell culture media with 10% fetal bovine serum for 3 days in the absence (unstimulated) and presence (stimulated) of 10 ng/mL of human IL-4. Both unstimulated and stimulated mouse lung supernatants were measured in duplicate at a 1:20 dilution. The Raw O.D. values for each sample are graphed, with the background O.D. shown as the dashed line. The stimulated mouse lung supernatants diluted 1:20 measured at 1,207 pg/mL, whereas the unstimulated mouse lung supernatants diluted 1:20 measured below the limit of detection of the assay.

17. ASSAY SPECIFICITY

This kit recognizes both native and recombinant mouse Eotaxin-2 protein in serum, plasma, and cell culture samples only.

18. SPECIES REACTIVITY

This kit recognizes mouse Eotaxin-2 protein.

Other species reactivity was determined by measuring 100% serum samples of various species, interpolating the protein concentrations from the Mouse standard curve, and expressing the interpolated concentrations as a percentage of the protein concentration in Mouse serum assayed at the same dilution.

Reactivity < 3% was determined for the following species:

- Human
- Rat
- Hamster
- Guinea Pig
- Rabbit
- Dog
- Goat
- Pig
- Cow

Please contact our Technical Support team for more information

19. TROUBLESHOOTING

Problem	Cause	Solution
Poor standard curve	Inaccurate Pipetting	Check pipettes
	Improper standard dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Incubation times too brief	Ensure sufficient incubation times; increase to 2 or 3 hour standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
	Incubation times with TMB too brief	Ensure sufficient incubation time until blue color develops prior addition of Stop solution
Large CV	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions.
	Contaminated wash buffer	Prepare fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	Store your reconstituted standards at -80°C, all other assay components 4°C. Keep TMB substrate solution protected from light.
Precipitate in Diluent	Precipitation and/or coagulation of components within the Diluent.	Precipitate can be removed by gently warming the Diluent to 37ºC.

Technical Support

For all technical or commercial enquiries please go to:

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