SensoLyte® Anti-Human MOG (1-125) Mouse IgG Specific Quantitative ELISA Kit

Colorimetric

Revision number: 1.2 *Last updated: 05/23/2014*

Catalog #	55153-M
Kit Size	One 96-well strip plate

This kit is optimized to detect anti-human MOG (1-125) IgG in mouse serum or cerebrospinal fluid. Wells are pre-coated with recombinant human MOG (1-125) protein and pre-blocked with BSA. The amount of anti-human MOG IgG in serum or cerebrospinal fluid is quantified using ELISA. Mouse anti-human MOG IgG standard is included. Ample materials and reagents are provided to perform 96 assays.

- ***Convenient Format***
  - Pre-coated and pre-blocked 96-well strip plate
  - Ready-to-use substrate solution and other assay components
  - 2-3 hours assay time at room temperature
- ***Minimal Sample Size***
  - Requires only 0.5-1 µl of serum or cerebrospinal fluid to perform assay
- ***High Sensitivity***
  - Detects as low as 1 ng/ml anti-human MOG (1-125) IgG
- ***Broad Dynamic Range***
  - 8-500 ng antibody/ml serum

Kit Components and Handling

Component	Description	Quantity
Component A	Human-MOG (1-125) coated and BSA blocked 8-well strips	12 strips
Component B	Mouse Anti-Human MOG (1-125) IgG standard	100 µl (20 µg/ml)
Component C	1X Sample Dilution Buffer	30 ml
Component D	10X Wash Buffer	50 ml
Component E	TMB color substrate solution	10 ml
Component F	Stop Solution	10 ml
Component G	Secondary antibody, Goat anti-Mouse IgG-HRP	30 µl (0.1mg/ml)

Other Materials Required (but not provided)

- Microplate reader: Capable of reading absorbance at 450 nm
- Rocking platform or shaker
- Strip ejector (to eject strips for later assay if not all strips are used in one experiment)
- Computer software: Capable of plotting Four Parameter Logistic Curve Fit (4-PL) (optional)
- Plate washer (optional)

Shipment and Storage

- Kit is shipped on blue ice. Upon receipt, store all kit components at 2-8°C for up to 12 months.

For Research Use Only. Not for Diagnostics.

Introduction

Myelin oligodendrocyte glycoprotein (MOG) is a member of the immunoglobulin superfamily and is expressed exclusively in the central nervous system.[1-3] Human MOG (1-125) is able to induce autoantibody production and relapsing-remitting neurological disease causing extensive plaque-like demyelination.[1-3] Autoantibody response to human MOG (1-125) has been observed in induced experimental autoimmune encephalomyelitis (EAE) in DA and Lewis rats, C57/BL6 and SJL mice, and common marmoset.[1-3] However, the exact pathological role and action of anti-human MOG (1-125) autoantibody is not known and is currently under vigorous investigation.[1-3]

The SensoLyte® Anti-Human MOG (1-125) Mouse IgG Specific Quantitative ELISA Kit provides a convenient quantitative assay for anti-human MOG (1-125) autoantibody. This kit is useful to researchers for determining the amount of anti-human MOG (1-125) antibody present, and can help provide information on the role it plays in the development and treatment of multiple sclerosis and for EAE, an animal model for multiple sclerosis pathogenesis.

Protocol

Please Note:

a) Allow kit components to warm up to room temperature before starting the assay
b) Spin down all components with volume less than 100 µl before use
c) Mix thoroughly washing buffer before diluting to dissolve any precipitated salt
d) More Sample Dilution Buffer can be made by adding 1% BSA into 1 X Wash Buffer

1. ELISA assay:

1.1 Establish dilution range of serum samples: Serial dilutions of serum samples can start from 1:1k, 1:5k, 1:25k, 1:125k. Use 1X Sample Dilution Buffer (Component C) to do the dilution (an example is shown in Table 1). Depending on the amount of antibody present, the dilution range can be further adjusted.

1.2 Arrange and label strips (Component A) based on the number of wells with standard and samples. An example is shown in Table 1. Although diluted standard and samples can be run as single points, duplicates are recommended.

Table 1. An example of four samples layout in duplicates using 6 strips.

	Standard [ng/ml]	**Standard [ng/ml]**	3	4	5	6
A	500	500	1:1K	1:1K	1:1K	1:1K
B	250	250	1:5K	1:5K	1:5K	1:5K
C	125	125	1:25K	1:25K	1:25K	1:25K
D	62.5	62.5	1:125K	1:125K	1:125K	1:125K
E	31.25	31.25	1:1K	1:1K	1:1K	1:1K
F	15.625	15.625	1:5K	1:5K	1:5K	1:5K
G	7.8125	7.8125	1:25K	1:25K	1:25K	1:25K
H	Blank	Blank	1:125K	1:125K	1:125K	1:125K

1.3 Dilute anti-human MOG (1-125) IgG standard (Component B) with 1X Sample Dilution Buffer (Component C) according to the Table 2.

Table 2. Serial dilution of mouse anti-human MOG (1-125) IgG standard.

Step	Concentration [ng/ml]	Anti-MOG IgG standard	Sample Dilution Buffer (Component C)
1	500.00	25 µl **Component B**	975 µl
2	250.00	500 µl from step 1	500 µl
3	125.00	500 µl from step 2	500 µl
4	62.5	500 µl from step 3	500 µl
5	31.25	500 µl from step 4	500 µl
6	15.625	500 µl from step 5	500 µl
7	7.812	500 µl from step 6	500 µl

1.4 Add 100 µl of the diluted standards and 100 µl of 1X Sample Dilution Buffer (Component C) as a blank into appropriate wells.

1.5 Add diluted samples into appropriate wells (depends on the number of samples to be tested). After adding the standards and samples to the wells, cover the plate and incubate at room temperature for 60 min with gentle shaking.

1.6 Prepare 1X working wash buffer by diluting the 10X Wash Buffer (Component D) with di H_2O.

1.7 Wash wells five times at 250 µl/well of 1X washing buffer. Pat dry.

1.8 Dilute goat anti-Mouse IgG-HRP (Component G) secondary antibody (2^{nd} Ab) with Sample Dilution Buffer (Component C): working solution at 1:2,000 dilution (based on 0.1 mg/ml concentration). Add 100 µl of the diluted 2^{nd} Ab into each well; incubate plate at room temperature for 45-60 min with gentle shaking.

1.9 Wash wells five times with 200 µl per well of 1X wash buffer. Pat dry. Clean the outside bottom of the wells with lens paper if necessary before the next step (this ensures accurate absorbance reading).

1.10 Add 100 µl of the TMB color substrate solution (Component E) into each well. Tap plate gently and incubate at room temperature until blue gradient is clearly observed across the wells (1-15 min). It may be necessary to adjust color development time so that absorbance values are within the detection range.

1.11 Add 50 µl of the Stop Solution (Component F) into each well and tap plate gently (blue color will turn to yellow). Measure absorbance (OD) at 450 nm using a microplate absorbance reader within 20 minutes after adding stop solution.

2. Calculate concentration of the samples.

2.1 Determine the average values (if replicates are used) for the standard and sample absorbance readings. Plot calibration curve using Four Parameter Logistic (4-PL) curve-fit. R^2 should be higher than 0.98. There should be at least 5 standard concentrations in the calculation to ensure statistical significance.

2.2 Choose absorbance values for the samples that are within the range used in the standard curve, and calculate the concentration of mouse anti-human MOG (1-125) IgG using 4-PL curve-fit.

2.3 Example of calculation of mouse and rat anti-human MOG (1-125) IgG concentrations:

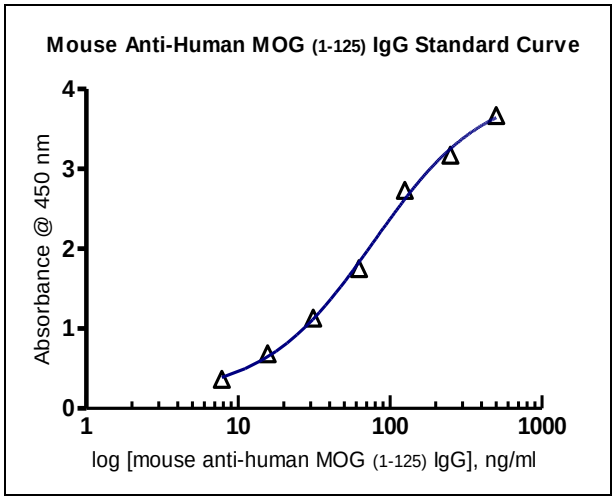
Please note, new standard curve must be generated each time the assay is run!

Table 3. An example of the assay with 4 samples using mouse ant-human MOG (1-125)IgG standard.

	1	2	3	4	5	6
A	3.538	3.801	2.588	2.339	2.576	2.622
B	3.273	3.067	**0.975**	**0.892**	**1.044**	**1.101**
C	2.749	2.713	0.280	0.237	0.309	0.351
D	1.823	1.677	0.087	0.071	0.087	0.104
E	1.170	1.095	2.686	2.597	2.297	2.469
F	0.705	0.668	**1.413**	**1.209**	**0.999**	**1.089**
G	0.366	0.367	0.544	0.459	0.367	0.379
H	0.025	0.018	0.175	0.147	0.114	0.136

Note: *Columns 1 and 2 are duplicate mouse IgG standards 500, 250, 125, 62.5, 31.25, 15.625, 7.8, and 0 ng/ml (Row A ~ H). Mouse Samples: Sample-1, 3A-D and 4A-D; Sample-2, 5A-D and 6A-D; Sample-3, 3E-H and 4E-H; Sample-4, 5E-H and 6E-H (at 1:1k, 5k, 25k, and 125k dilution in duplicates the same as in Table 1).Values jn bold were used for calculations.*

2.3.1 Four-parameter logistic curve-fit (4-PL) based on the average absorbance reading values:



Y=[(A-D)/(1+{x/C}^B)]+D, A=0.1647 B=1.206 C=78.15 D=4.013 R^2=0.997

2.3.2 From 4-PL curve-fit data table (not shown) generated by computer software the following concentrations for mouse samples were obtained (based on the average absorbance readings):

	Absorbance@ 450nm, mean value	Calculated Concentration [ng/ml]	Dilution Factor	Adjusted Serum Concentration, [mg/ml]
Sample1	0.933	24.66	1:5000	0.123
Sample2	1.072	29.81	1:5000	0.149
Sample3	1.311	39.02	1:5000	0.195
Sample4	1.044	29.02	1:5000	0.145

References:

1. Von Büdingen, H-C. et al. (2001) *J. Clin. Immunol.* 21 (3): 155-170.
2. Marta C.B. et al. (2005) *PNAS* 102 (39): 13992-13997.
3. Lyons, J-A. et. al. (1999) *European Journal of Immuology* 29: 3432-3439