# SensoLyte™ Anti-Rat MOG (1-125) IgG Quantitative ELISA Kit
## *Colorimetric*

*Revision number: 1.4* *Last updated: 03/04/2016*

| Catalog # | AS-55157 |
|---|---|
| Kit Size | One 96-well strip plate |

This kit is optimized to detect anti-rat MOG (1-125) IgG. Wells are pre-coated with recombinant rat MOG (1-125) protein and pre-blocked with BSA. The amount of anti-rat MOG IgG in serum or cerebrospinal fluid is quantified using ELISA. 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-rat MOG (1-125) IgG
- ***Broad Dynamic Range***
  - 8-500 ng antibody/ml serum (depending on colorimetric developing time)

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### Kit Components and Handling

| Component | Description | Quantity |
|---|---|---|
| Component A | Rat-MOG (1-125) coated and BSA blocked 8-well strips | 12 strips |
| Component B | Anti-Rat 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-Rat IgG-HRP | 30 µl |

### 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)

### 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.[1-5] Rat MOG (1-125) is able to induce autoantibody production and relapsing-remitting neurological disease causing extensive plaque-like demyelination.[1-5] Autoantibody response to rat 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.[1-4] However, the exact pathological role and action of anti-rat MOG (1-125) autoantibody is not known and is currently under vigorous investigation.[1-5]

The SensoLyte™ Anti-Rat MOG (1-125) IgG Quantitative ELISA Kit provides a convenient quantitative assay for anti-rat MOG (1-125) autoantibody. This kit is useful to researchers for determining the amount of anti-rat MOG (1-125) antibody present, and can help provide information on the role it plays in the development and treatment of 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 well 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-rat MOG (1-125) IgG standard (Component B) in 1X Sample Dilution Buffer (Component C) according to the Table 2.

**Table 2**. Serial dilution of anti-rat MOG (1-125) IgG standard.

| Step | Concentration [ng/ml] | Anti-MOG IgG standard (Component B) | Sample Dilution Buffer (Component C) |
|---|---|---|---|
| 1 | 500.00 | 25 µl from the stock | 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 into wells (A1-G2 for duplicate run). Add 100 µl of 1X Sample Dilution Buffer (Component C) as a blank into wells H1-H2.

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 200 µl/well of 1X washing buffer. Pat dry.

1.8 Dilute goat anti-Rat IgG-HRP (Component G) secondary antibody ($2^{nd}$ Ab) with sample dilution buffer (component C): working solution at 1:2,000 dilution. 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 washing 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 fall 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 the 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 0.99. 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 anti-rat MOG (1-125) IgG using 4-PL curve-fit.

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

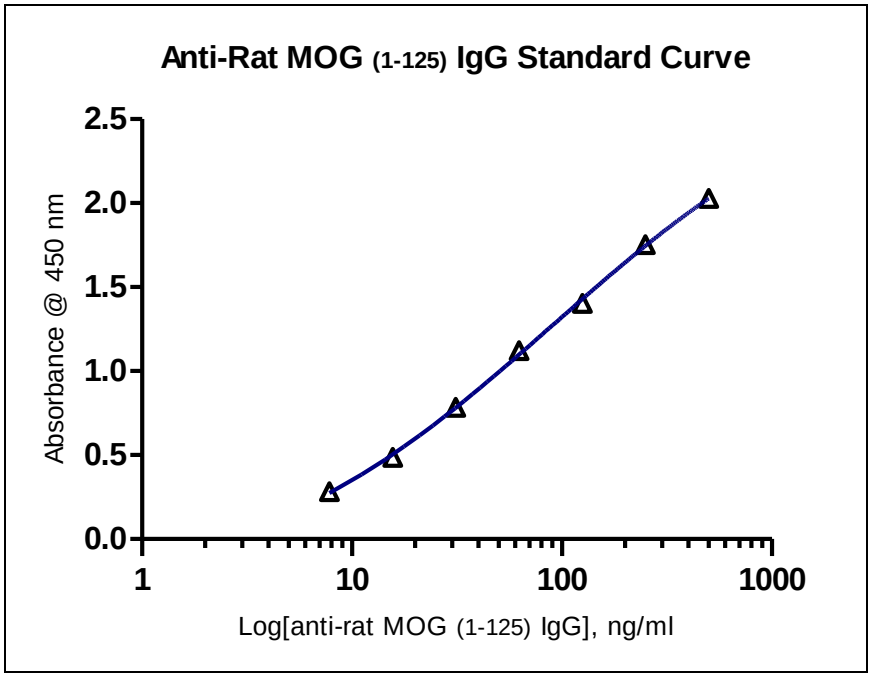
**Please note, new standard curve must be generated each time the assay is run.**

**Table 3.** An example of the assay with 4 samples.

| | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| A | 2.093 | 1.964 | 1.999 | 2.021 | **1.544** | **1.540** |
| B | 1.827 | 1.682 | **1.576** | **1.503** | 0.823 | 0.867 |
| C | 1.416 | 1.393 | 0.959 | 0.948 | 0.292 | 0.287 |
| D | 1.179 | 1.066 | 0.431 | 0.421 | 0.088 | 0.091 |
| E | 0.836 | 0.735 | 1.628 | 1.513 | **1.189** | **1.244** |
| F | 0.527 | 0.445 | **1.011** | **0.951** | 0.563 | 0.612 |
| G | 0.311 | 0.256 | 0.448 | 0.397 | 0.219 | 0.224 |
| H | 0.031 | 0.022 | 0.131 | 0.129 | 0.079 | 0.079 |

*Note: Columns 1 and 2 are duplicate standards 500, 250, 125, 62.5, 31.25, 15.625, 7.8, and 0 ng/ml (Row A ~ H). 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). Values in 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=-03259 B=0.5975 C=90.7 D=2.877 $R^2$=0.9994

2.3.2 From 4-PL curve-fit data table (not shown) generated by computer software the following

concentrations for samples were obtained (based on the average absorbance readings):

| | Absorbance @ 450nm, mean value | Calculated Concentration [ng/ml] | Dilution Factor | Actual Sample Concentration [mg/ml] |
|---|---|---|---|---|
| **Sample1** | 1.5395 | 158.5 | 1:5000 | 0.79 |
| **Sample2** | 1.542 | 159 | 1:1000 | 0.159 |
| **Sample3** | 0.981 | 48.2 | 1:5000 | 0.241 |
| **Sample4** | 1.2165 | 80.1 | 1:1000 | 0.081 |

**References:**


1. Von Büdingen, H-C. et al. (2001) *Journal of Clinical Immunology* 28 (3): 155-170
2. Adelmann, M. et al. (1995) *Journal of Neuroimmunology* 63: 17-27
3. Oliver, R. A. et al. (2003) *The Journal of Immunology* 171: 462-468
4. Weissert, R. et al. (1998) *Journal of Clinical Investigations* 102 (6): 1265-1273
5. Johns, T. G. et al. (1995) *The Journal of Immunology* 154: 5536-5541.