SensoLyte® Anti-PLP (139-151) IgG Quantitative ELISA Kit (Mouse) *Colorimetric*

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

Catalog #	55524
Kit Size	One 96-well strip plate

This kit is optimized to detect mouse anti-PLP (139-151) IgG. Wells are pre-coated with PLP (139-151) peptide and pre-blocked with BSA. The amount of anti-PLP (139-151) IgG in mouse 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 100 pg anti-PLP (139-151) IgG
  - No cross-reactivity with anti-PLP (178-191) IgG
- ***Broad Dynamic Range***
  - 8-500 ng antibody/ml serum (depending on colorimetric developing time)

Kit Components and Handling

Component	Description	Quantity
Component A	PLP (139-151) coated and BSA blocked 8-well strips	12 x 8 strips
Component B	Mouse anti-PLP (139-151) 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 (0.1 mg/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 future 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 with blue ice. Store all kit components at 2-8°C for up to 12 months.

Introduction

Proteolipid protein (PLP) is the most abundant protein in the central nervous system (CNS) myelin sheath and is highly conserved among species.[1-2] PLP has a 50% hydrophobic amino acids content and a molecular weight of 30 kDa[1]. Mouse PLP (139-151) is able to induce autoantibody production and relapsing-remitting neurological disease causing extensive plaque-like demyelination.[1-4] Autoantibody response to mouse PLP (139-151) has been observed in induced experimental autoimmune encephalomyelitis (EAE) in SJL, F1, and SWR mice strains.[3-4] However, the exact pathological role and action of anti-mouse PLP (139-151) autoantibody is not well understood.[1-4]

The SensoLyte® Anti-PLP (139-151) IgG Quantitative ELISA Kit (mouse) provides a convenient and quantitative assay for anti-PLP (139-151) autoantibody in mouse. This kit is useful to researchers who wants to determine the amount of anti-PLP (139-151) antibody present in biological samples, and can help provide information on the role it plays in the development and treatment of EAE,[1-4] an animal model for MS 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 150 µl before use
c) Mix well 10X Washing Buffer before diluting to dissolve any precipitated salt
d) More Sample Dilution Buffer can be made by adding 0.1% BSA into 1 X Wash Buffer

1. ELISA assay:

1.1 Establish dilution range of mouse 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 dilute samples and standards (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 serum samples layout in duplicates using 6 eight-well 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 Mouse anti-PLP (139-151) IgG Standard (Component B) in 1X Sample Dilution Buffer (Component C) according to the Table 2.

Table 2. Serial dilution of anti-PLP (139-151) IgG standard.

Step	Concentration [ng/ml]	Anti-$PLP_{139-151}$ 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 $A_{1,2}$-$G_{1,2}$ for duplicate run). Add 100 µl of 1X Sample Dilution Buffer (Component C) as a blank into wells $H_{1,2}$.

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 with 200 µl per 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). Secondary antibody working solution is 1:2,000 dilution (based on 0.1 mg/ml concentration). Add 100 µl of the diluted 2^{nd} Ab into each well and 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 (1-10 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 serially diluted standard concentrations in the calculation to ensure statistical significance.

2.2 Choose absorbance values for the serum samples that are within the range used in the standard curve, and calculate the concentration of anti-PLP (139-151) IgG using 4-PL curve-fit.

2.3 Example of calculation of mouse anti-PLP (139-151) IgG concentrations:

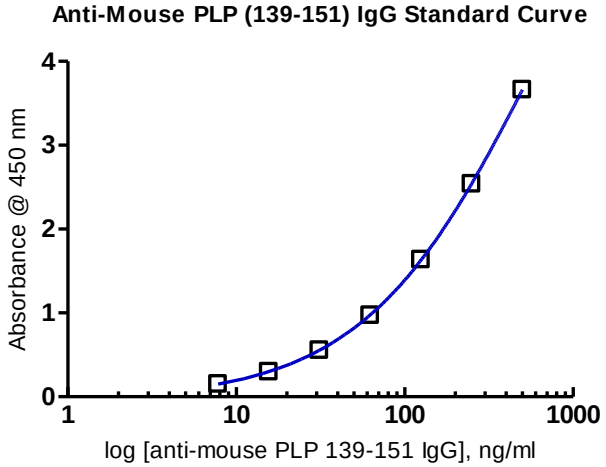
Please note, new standard curve must be generated each time an assay is run.

Table 3. An example of the assay with 5 serum samples using mouse anti-PLP (139-151) IgG standard.

	1	2	3	4	5	6	7	8
A	3.716	3.609	4.147	4.058	3.256	3.357	4.037	3.913
B	2.577	2.501	**1.790**	**1.783**	**1.036**	**1.027**	**1.690**	**1.776**
C	1.640	1.630	0.526	0.536	0.228	0.240	0.700	0.603
D	1.001	0.944	0.161	0.161	0.066	0.064	0.156	0.177
E	0.572	0.540	4.080	4.110	3.549	3.373	0.030	0.028
F	0.311	0.288	**2.218**	**2.095**	**1.541**	**1.401**	0.017	0.017
G	0.160	0.144	0.712	0.776	0.478	0.461	0.017	0.017
H	0.014	0.010	0.192	0.206	0.119	0.132	0.016	0.015

Note: *Columns 1 and 2 are duplicate mouse anti-PLP (139-151) 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, 7A-D and 8A-D; Sample-4, 3E-H and 4E-H; Sample 5, 5E-H and 6E-H; Negative Control serum, 7E-H and 8 E-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=-0.04 B=0.8577 C=584.1 D=7.891 R^2=1.0

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

anti-PLP (139-151) concentrations for mouse 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	1.786	143.2	1:5000	0.716
Sample2	1.031	67.23	1:5000	0.336
Sample3	1.733	136.7	1:5000	0.683
Sample4	2.156	191.5	1:5000	0.957
Sample5	1.471	108.7	1:5000	0.543

References:

1. Tzakos, A. G. et al. *Curr. Med.Chem.***12** (2005): 1569-1587
2. Miller, D. et al. *Curr. Protocols Immunol.* (2007): 15.1.1-15.1.18
3. Tuohy, V. et al. *J. Neuroimmunol* **39** (2003) : 67-74
4. Kuchroo, V. et al. *Pathobiol* **59** (1991) : 305-312