Creative Diagnostics®

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User's Manual

Anti-Human MOG (1-125) Human IgG Specific ELISA Kit

REF **DEIABL465**

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

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

This kit is specifically designed for the detection of anti-human MOG (1-125) IgG in human samples.

General Description

Myelin oligodendrocyte glycoprotein (MOG) is an immunoglobulin superfamily member found solely within the central nervous system. The human MOG (1-125) peptide is known to trigger autoantibody generation, resulting in a relapsing-remitting neurological condition accompanied by widespread demyelination resembling plaques. This specific autoantibody response has been observed in experimental autoimmune encephalomyelitis (EAE) models utilizing DA rats, Lewis rats, C57/BL6 mice, SJL mice, and common marmosets. The exact pathological function of anti-human MOG (1-125) autoantibodies is not yet fully defined and is a subject of intense research.

The Anti-Human MOG (1-125) Human IgG ELISA Kit provides an efficient solution for the precise measurement of these autoantibodies in human serum or plasma samples. It aids researchers in determining antibody levels and contributes to understanding their involvement in the development and management of multiple sclerosis.

Principles of Testing

The assay employs microplate wells that are pre-coated with the MOG (1-125) peptide and pre-blocked with proprietary solution, facilitating the quantification of anti-MOG (1-125) IgG in serum or plasma via ELISA. The kit contains sufficient materials and reagents to perform a total of 96 assays.

Reagents And Materials Provided

1. Human-MOG (1-125) coated and blocked 8-well strips, 12 strips
2. Human anti-Human MOG (1-125) IgG standard, 500 µl (100ng/ml) × 3
3. 1× Sample Dilution Buffer, 30 ml
4. 10× Wash Buffer, 50 ml
5. TMB color substrate solution, 10 ml
6. Stop Solution, 10 ml
7. Secondary antibody, Goat anti-Human IgG-HRP, 30 µl

Materials Required But Not Supplied

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

5. Plate washer (optional)

Storage

All components except the standards should be stored at 2-8°C for up to 12 months, while the standards should be stored at -20°C.

Assay Procedure

Important Notes Prior to Assay

1. Equilibrate all kit components to room temperature.
2. Briefly centrifuge all liquid components in volumes under 100 µl.
3. Thoroughly mix the Washing Buffer before dilution to ensure any crystallized salts are fully dissolved.
4. Additional Sample Dilution Buffer can be prepared by supplementing 1× Wash Buffer with 1% BSA.

Procedure

1. Prepare Sample Dilutions: Dilute human serum or plasma samples at a recommended starting ratio of 1:40 using 1× Sample Dilution Buffer. The dilution factor can be optimized based on the expected antibody concentration.
2. Layout Microplate Strips: Arrange and label the pre-coated strips according to the number of standard and sample wells required. The use of duplicate wells for both standards and samples is advised (see Table 1 for an example).

Table 1. An example of four samples layout in duplicates using 3 strips.

	Standard [ng/ml]	Standard [ng/ml]	3
A	100	100	Sample A 1:40
B	50	50	Sample A 1:40
C	25	25	Sample B 1:40
D	12.5	12.5	Sample B 1:40
E	6.25	6.25	Sample C 1:40
F	3.125	3.125	Sample C 1:40
G	1.56	1.56	Sample D 1:40
H	Blank	Blank	Sample D 1:40

3. Prepare Standard Dilutions: Generate a series of standard dilutions by diluting the anti-human MOG (1-125) IgG standard with 1× Sample Dilution Buffer, following the scheme provided in Table 2.

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

Step	Concentration [ng/ml]	Anti-MOG IgG standard	Sample Dilution Buffer
1	100	Human anti-Human MOG (1-125) IgG standard	0
2	50	250 µl from step 1	250 µl
3	25	250 µl from step 2	250 µl
4	12.5	250 µl from step 3	250 µl
5	6.25	250 µl from step 4	250 µl
6	3.125	250 µl from step 5	250 µl
7	1.56	250 µl from step 6	250 µl

4. Load Standards and Blank: Dispense 100 µl of each diluted standard into the appropriate duplicate wells. Add 100 µl of 1× Sample Dilution Buffer into the designated wells to serve as the blank.
5. Load Samples and First Incubation: Pipette 100 µl of each diluted sample into its assigned wells. Once loaded, seal the plate and incubate at room temperature for 60 minutes with gentle agitation.
6. Prepare Wash Buffer: Dilute the provided 10× Wash Buffer with deionized water to prepare a 1× working solution.
7. First Wash Cycle: Wash each well four times using 250 µl of the 1× wash buffer per cycle. After the final wash, remove residual liquid by tapping the plate onto absorbent paper.
8. Secondary Antibody Incubation: Prepare a 1:2,000 working dilution of the Goat anti-Human IgG-HRP conjugate in Sample Dilution Buffer. Add 100 µl to each well, cover the plate, and incubate at room temperature with gentle shaking for 45 to 60 minutes.
9. Second Wash Cycle: Repeat the washing procedure five times with 250 µl of 1× wash buffer per well. Pat the plate dry and, if necessary, wipe the bottom surface with lens paper to ensure accurate absorbance readings.
10. Color Development: Add 100 µl of TMB Substrate Solution to each well. Tap the plate gently to mix and incubate at room temperature. Monitor development until a distinct blue color gradient appears (typically 1-5 minutes). The incubation time may be adjusted to ensure optical density values fall within the assay's dynamic range.
11. Reaction Stop and Reading: Stop the enzymatic reaction by adding 50 µl of Stop Solution to each well (color changes to yellow). Measure the absorbance (OD) at 450 nm using a microplate reader within 20 minutes.

Calculation

1. First, calculate the average absorbance from any replicate measurements for both the standard and sample wells. Then, generate a standard calibration curve by applying a Four Parameter Logistic (4-PL) curve fit to the standard data. For a reliable curve, the coefficient of determination (R^2) should exceed 0.98, and data from a minimum of five standard concentrations must be included to ensure statistical validity.
2. Finally, select sample absorbance readings that fall within the dynamic range of the standard curve. Use the 4-PL curve-fit model to interpolate and calculate the corresponding concentration of anti-human MOG (1-125) IgG in each sample.