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User's Manual

Anti-MOG (35-55) IgG ELISA Kit

REF **DEIA6141**

Σ **96T**

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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-MOG (35-55) IgG antibodies derived from mice or rats.

General Description

Myelin oligodendrocyte glycoprotein (MOG), a member of the immunoglobulin superfamily, is found exclusively within the central nervous system. The MOG (35-55) peptide can trigger the production of autoantibodies, leading to a relapsing-remitting neurological disorder characterized by widespread plaque-like demyelination. Such an autoantibody response to MOG (35-55) has been detected not only in patients with multiple sclerosis (MS) but also in C57/BL6 mice and Lewis rats with MOG (35-55)-induced experimental autoimmune encephalomyelitis (EAE). The Anti-MOG (35-55) IgG ELISA Kit (mouse/rat) offers a convenient and quantitative method for detecting these autoantibodies in murine and rat samples. It serves as a valuable tool for researchers to determine antibody levels and investigate their role in the pathogenesis and potential treatment of EAE, a key animal model for studying MS.

Principles of Testing

The assay employs microplate wells that are pre-coated with the MOG (35-55) peptide and pre-blocked with BSA, facilitating the quantification of anti-MOG (35-55) IgG in serum or cerebrospinal fluid samples via ELISA. The kit contains sufficient materials and reagents to perform a total of 96 assays.

Reagents And Materials Provided

1. MOG (35-55) coated and BSA blocked 8-well strips, 12 strips
2. Mouse anti-MOG (35-55) IgG standard, 110 µl (5 µg/ml)
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-Mouse IgG-HRP, 30 µl
8. Secondary antibody, Goat anti-Rat IgG-HRP, 30 µl
9. Rat anti-MOG (35-55) IgG standard, 110 µl (5 µg/ml)

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 future assay if not all strips are used in one experiment)

4. Computer software: Capable of plotting Four Parameter Logistic Curve Fit (4-PL) (optional)

Storage

All components should be stored at 2-8°C for up to 12 months.

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 10× 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. Establish Sample Dilutions: Prepare serial dilutions of serum samples starting at 1:1,000, 1:5,000, 1:25,000, and 1:125,000 using 1× Sample Dilution Buffer. The dilution range can be optimized based on the expected antibody concentration. See Table 1 for an example layout.
2. Prepare the Plate: Arrange and label the pre-coated strips according to the planned assay layout (Table 1). Running duplicates for both standards and samples is recommended.

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

	Standard [ng/ml]	**Standard [ng/ml]**	3	4	5	6
A	500	500	1:1,000	1:1,000	1:1,000	1:1,000
B	250	250	1:5,000	1:5,000	1:5,000	1:5,000
C	125	125	1:25,000	1:25,000	1:25,000	1:25,000
D	62.5	62.5	1:125,000	1:125,000	1:125,000	1:125,000
E	31.25	31.25	1:1,000	1:1,000	1:1,000	1:1,000
F	15.625	15.625	1:5,000	1:5,000	1:5,000	1:5,000
G	7.8125	7.8125	1:25,000	1:25,000	1:25,000	1:25,000
H	Blank	Blank	1:125,000	1:125,000	1:125,000	1:125,000

3. Prepare Standards: Dilute either the mouse or rat anti-MOG (35-55) IgG standard in 1× Sample Dilution Buffer following the serial dilution scheme in Table 2.

Table 2. Serial dilution of anti-MOG (35-55) IgG standard.

Step	Concentration [ng/ml]	Mouse/Rat Anti-MOG IgG standard	Sample Dilution Buffer
1	500.00	100 µl from the stock	900 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

4. Load the Plate: Pipette 100 µl of each diluted standard into the designated duplicate wells (e.g., A1,2 through G1,2). Add 100 µl of 1× Sample Dilution Buffer to wells H1,2 as the blank. Dispense diluted samples into their assigned wells. Seal the plate and incubate at room temperature with gentle shaking for 60 minutes.
5. Prepare Wash Buffer: Dilute the 10× Wash Buffer with deionized water to make 1× working solution.
6. Wash the Plate: Wash each well five times with 200 µl of 1× wash buffer. After the final wash, remove residual liquid by tapping the plate onto absorbent paper.
7. Add Secondary Antibody: Prepare a 1:2,000 dilution of the appropriate HRP-conjugated secondary antibody (Goat anti-Mouse IgG-HRP for mouse samples; Goat anti-Rat IgG-HRP for rat samples) in Sample Dilution Buffer. Add 100 µl to each well. Cover and incubate at room temperature with gentle shaking for 45–60 minutes.
8. Wash Again: Repeat the wash procedure (Step 6) five times. Pat the plate dry. Before proceeding, wipe the bottom of the plate with lens paper if necessary to ensure accurate optical readings.
9. Color Development: Add 100 µl of TMB Substrate Solution to each well. Tap the plate gently and incubate at room temperature until a clear blue gradient appears (typically 1–15 minutes; development time may require optimization to ensure readings are within the dynamic range).
10. Stop Reaction and Read: Add 50 µl of Stop Solution to each well and tap gently (color changes to yellow). Measure the absorbance (OD) at 450 nm using a microplate reader within 20 minutes of adding the stop solution.

Calculation

Calculate the average absorbance from replicates for standards and samples. Construct the standard calibration curve using a Four Parameter Logistic (4-PL) model, ensuring an $R^2 > 0.99$ and incorporating data from a minimum of five standard dilution points for statistical reliability. Sample absorbance readings must be within the standard curve's range. Use the 4-PL fit to calculate the anti-MOG (35-55) IgG concentration from these absorbance values.