Version 13-06/24

User's Manual

Human IgG subclass ELISA kit

REF **DEIA9474**

Σ **2×96T**

RUO

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

The Human IgG Subclass ELISA contains components required to construct an enzyme-linked immunoassay for the specific and quantitative measurement of Human IgG1, IgG2, IgG3, and IgG4 subclasses.

General Description

The Human IgG subclass ELISA Kit contains components required to construct an enzyme-linked immunoassay for the specific and quantitative measurement of Human IgG1, IgG2, IgG3, and IgG4 subclasses. Sufficient quantities of reagents are provided to yield 2 plates of 96 wells if the recommended assay procedure, storage and handling of materials are followed as specified on this insert. This kit is a sandwich-type ELISA using a horseradish peroxidase detection system. A coated microtiter plate captures monoclonal reagents that are specific to the various human IgG subclasses. The monoclonal antibodies in turn capture the human IgG subclasses, for which they are specific, out of the serum sample. These monoclonal antibodies have been characterized in an IUIS/WHO study. The captured human IgG is then labeled by a horseradish-peroxidase anti-human IgG reagent. The detection signal is then generated in proportion to the amount of human subclass antibody.

Principles of Testing

This kit is a sandwich type ELISA using a horseradish peroxidase detection system. A coated microtiter plate captures monoclonal reagents which are specific to the various human IgG subclasses. The monoclonal antibodies in turn capture the human IgG subclasses, for which they are specific, out of the serum sample. These monoclonal antibodies have been characterized in a IUIS/WHO study. The captured human IgG is then labeled by a horseradish-peroxidase anti-human IgG reagent. The detection signal is then generated in proportion to the amount of human subclass antibody.

Reagents And Materials Provided

1. mAb Anti-Human IgG1, mAb Anti-Human IgG2, mAb Anti-Human IgG3, mAb Anti-Human IgG4: Storage: 2-8°C until expiration date. 4 vials × 2.5 mL.

2. Human Serum Control: 2 vials. Contains 0.1% sodium azide. Storage: 2-8°C until expiration date. Reconstitute the lyophilized control with Diluent Buffer.

3. Human IgG Subclass Standard: 2 vials. Contains 0.1% sodium azide. Storage: 2-8°C until expiration date.

4. Peroxidase Anti-Human IgG: 0.5 mL (50× concentrate). Storage: 2-8°C until expiration date.

5. TMB Solution: 25 mL

6. Stop Solution: 25 mL

7. Diluent Buffer: 135 mL

8. Wash Buffer Concentrate (25×): 100 mL.

9. Antibody-Coated Wells: 12 × 8 Well Strips, 2 Plates

Materials Required But Not Supplied

1. Pipettes and timer
2. Microplate reader with a detector that can measure absorbance at 450 nm
3. 1 L graduated cylinder, plate washer or wash bottles
4. Polypropylene tubes for standards and sample dilutions, if needed

Storage

2°C to 8°C

Reagent Preparation

1. Human Serum Control: Reconstitute the lyophilized control with Diluent Buffer. Reconstitution volume is stated on the label of the control vial. Swirl or mix gently and allow to sit for 10 minutes to ensure complete reconstitution. Use control within 1 hour of reconstitution.

2. Human IgG Subclass Standard: Reconstitute the lyophilized standard with 1.0 mL of Diluent Buffer. Swirl or mix gently and allow to sit for 10 minutes to ensure complete reconstitution. Use standard within 1 hour of reconstitution. Standard Curve: To generate a 6-point standard curve, make serial dilutions of the standard using Diluent Buffer. **Note: For standard concentrations see CoA.**

3. Peroxidase Anti-Human IgG: Recommended dilution: Dilute concentrated Peroxidase Anti-Human IgG in Diluent Buffer at a ratio of 1:50. For example, add 0.22 mL of conjugate to 10.78 mL of diluent for each 96 well plate. Do not prepare more diluted Anti-Human IgG solution than is needed. Discard any unused portion.

4. Wash Buffer Concentrate (25 ×): Dilute 1 volume of Wash Buffer Concentrate (25×) with 24 volumes of deionized water (i.e., 100 mL may be diluted up to 2.5 L).

Assay Procedure

1. Prior to use, allow the kit to warm to room temperature. Remove the number of strip-wells according to your design plan. It is suggested to run all samples in duplicate.

   Table 1 Example of experimental plate plan setup for IgG1 only:

0	0	Control	Control								
Neat	Neat	Sample	Sample								
1:2	1:2	Sample	Sample								
1:4	1:4	Sample	Sample								
1:8	1:8	Sample	Sample								
1:16	1:16	Sample	Sample								
1:32	1:32	Sample	Sample								
		Sample	Sample								

2. Add 50 µL of the appropriate human subclass specific antibody (e.g., mAb Anti-Human IgG1) to each well in

the strip.

3. For the zero wells, add 50 µL of Diluent Buffer. Add 50 µL of diluted serum samples, standards, and the ready-to-use Human Serum Control to their respective wells. (Suggested dilution for human sample is 1:2,500 as a starting point. However, it is up to the investigator to determine the optimal dilution.) Gently tap the plate on the side 10 times to mix. Incubate at room temperature for 30 min.
4. Remove contents from the plate by inversion or aspiration. Wash four times by adding 300 µL of diluted Wash Buffer into each well. Let soak for 10-15 seconds, then remove excess by inverting plate and tapping on absorbent paper to remove excess liquid.
5. Add 100 µL of diluted Peroxidase Anti-Human IgG solution into each well. Incubate at room temperature for 30 min.
6. Remove contents from the plate by inversion or aspiration. Wash four times using the method in Step 4.
7. Add 100 µL of the ready-to-use TMB Solution into each well. The liquid in the wells will begin to turn blue. Incubate at room temperature in the dark for 10 min.
8. Quickly add 100 µL Stop Solution into each well. Tap side of plate gently to mix. The solution in the wells should change from blue to yellow.
9. Measure absorbance at 450 nm (reference absorbance: 650 nm) within 1 hour of adding the Stop Soution. Calculate results using a log-log or 4-parameter curve fit.