Human IgG Subclass Profile

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Product description

The Human IgG Subclass Profile 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.

Materials provided

mAb Anti-Human IgG1 **mAb Anti-Human IgG2** **mAb Anti-Human IgG3** **mAb Anti-Human IgG4**	Storage: 2-8°C until expiration date. Quantity: 4 vials × 2.5 mL.
Human Serum Control	Quantity: 2 vials. Contains 0.1% sodium azide. Storage: 2-8°C until expiration date 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.
Human IgG Subclass Standard	Quantity: 2 vials. Contains 0.1% sodium azide. Storage: 2-8°C until expiration date 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.
Peroxidase Anti-Human IgG	Quantity: 0.5 mL (50X concentrate). Storage: 2-8°C until expiration date 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.
TMB Solution	Quantity: 25 mL
Stop Solution	Quantity: 25 mL
Diluent Buffer	Quantity: 135 mL
Wash Buffer Concentrate (25 X)	Quantity: 100 mL Dilute 1 volume of Wash Buffer Concentrate (25X) with 24 volumes of deionized water (i.e., 100 mL may be diluted up to 2.5 L).
Antibody-Coated Wells	Quantity: 12 × 8 Well Strips, 2 Plates

Additional materials required

- Pipettes and timer
- Microplate reader with a detector that can measure absorbance at 450 nm
- 1 L graduated cylinder [;ate washer or wash bottles
- Polypropylene tubes for standards and sample dilutions, if needed

Recommended 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.

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For descriptions of symbols on product labels or product documents, go to **thermofisher.com/symbols-definition**.