Human IgG Subclass Profile

2 Plate Format
Technical Data Sheet

Catalog #: 99-1000 Lot #: See product

Intended Use and Materials Provided

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 and recommended storage and handling of materials are followed as specified on this insert. Research Use Only.

1. **Antibody:** **MAb Anti-Human IgG1 (Part # 50270HK)**
   MAb Anti-Human IgG2 (Part # 50271HK)
   MAb Anti-Human IgG3 (Part # 50272HK)
   MAb Anti-Human IgG4 (Part # 50273HK)
   Form: Liquid, 4 vial X 2.5 mL each vial
   Storage: Store at 2 to 8ºC until expiration date.
2. **Control:** **Human Serum Control (Part # 50173)**
   Form: Lyophilized, 2 vials
   Storage: Store at 2 to 8ºC until expiration date.
   Recommended Dilution: Reconstitute the lyophilized control with 0.5mL of Diluent Buffer. Vortex or gently agitate to dissolve completely prior to use.
3. **Standard:** **Human IgG Subclass Standard (Part #50287HK)**
   Form: Lyophilized, 2 vials
   Storage: Store at 2 to 8ºC.
   Reconstitution: Reconstitute each lyophilized standard vial with 1.0mL of Diluent Buffer. Vortex or gently agitate to dissolve completely prior to use.
   Standard Curve: To generate a 6-point standard curve, make serial dilutions of the standard using the Diluent Buffer. When reconstituted in 1mL, the concentration of the standard is: 13.72 µg/mL of IgG1, 5.32 µg/mL of IgG2, 1.34 µg/mL of IgG3, and 0.76 µg/mL of IgG4. Below is the concentration of each IgG when diluted serially in half.

Standard (µg/mL)

	IgG1	IgG2	IgG3	IgG4
Neat	13.72	5.32	1.34	0.76
1:2	6.86	2.66	0.67	0.38
1:4	3.43	1.33	0.34	0.19
1:8	1.72	0.67	0.17	0.095
1:16	0.86	0.33	0.084	0.048
1:32	0.43	0.17	0.042	0.024

4. **Secondary antibody:** **Peroxidase Anti-Human IgG (Part #50177HK)**
   Form: Liquid, 1 vial x 0.5 mL (50X concentrate)
   Storage: Store at 2 to 8ºC until expiration date.
   Recommended Dilution: Dilute concentrated peroxidase-anti-human IgG in Sample Diluent at a ratio of 1:50. For example, add 0.2 ml of conjugate to 11 ml of diluent for each 96 well plate. Do not prepare more diluted Anti-Human IgG solution than is needed. Discard any unused portion.
5. **Chromogen:** **TMB Solution (Part # SB01)**
   Form: 1 vial X 25 mL
   Stop Solution: **Stop Solution (Part # SS01)**
   Form: 1 vial x 25 mL
6. **Diluent:** **Diluent Buffer (Part #50289HK)**
   Form: 1 vial x 135 mL

7. **Wash Buffer:** **Wash Buffer Concentrate (25X) (Part # WB01)**
   Form: 100mL bottle
   Reconstitution: Dilute 1 volume of the 25x wash buffer concentrate with 24 volumes of deionized water (ie. 100mL may be diluted up to 2.5 liters).
8. **Plate:** **Antibody Coated Wells - 2 plates (Part # 40150)**
9. **Protocol**

Additional Materials Required

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

Principle of the Assay

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.

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.

Example of experimental plate plan setup for IgG1 only:

Standard IgG1

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 (for example, *MAb Anti-Human IgG1*) to each well except for zero wells. For the zero wells, add 50 µl of diluted serum samples and then, add 50 µl of the *Diluent Buffer*
3. Then, *a*dd 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 1:2500 as a starting point. However, it is up to the investigator to determine the optimal dilution.) Incubate at room temperature for 30 min.
4. Remove contents inverting the plate into the sink Add 200 µl of diluted *Wash Buffer* into each well and remove by inverting the plate into the sink and tap on absorbent paper to remove access liquid. Repeat washes, three times.
5. Add 100 µl of diluted *Peroxidase Anti-Human IgG* conjugate solution into each well. Incubate at room temperature for 30 min.
6. Remove contents inverting the plate into the sink. Repeat washes as in Step 4, three times.
7. Add 100 µl of the ready-to-use *TMB Solution* into each well. Incubate at room temperature for 10 min.
8. Quickly add 100 µl of *Stop Solution* into each well and shake for a few seconds. A dramatic color change from blue to yellow should occur.
9. Measure absorbance at 450 nm (reference absorbance: 650 nm) within 1 hour of adding the *Stop Solution*. Calculate results using a log-log or 4-parameter curve fit.