Human IgG Subclass Profile

192 Tests
Technical Data Sheet

Catalog #: 991000

Lot #*: 1694083

*Note: A letter at the end of the lot number signifies an additional packaging of this same lot.

Product 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, storage and handling of materials are followed as specified on this insert.

1. **Antibody:** **mAb Anti-Human IgG1 (Part # 50270HK Lot #:** 1694084**)**
   mAb Anti-Human IgG2 (Part # 50271HK Lot #:1694085**)**
   mAb Anti-Human IgG3 (Part # 50272HK Lot #:1694086**)**
   mAb Anti-Human IgG4 (Part # 50273HK Lot #:1694087**)**
   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 Lot #: 1694091)**
   Form: Lyophilized, 2 vials. Contains 0.1% sodium azide.
   Storage: Store at 2 to 8ºC until expiration date.
   Reconstitution: Reconstitute the lyophilized control with 1.0 mL of Diluent Buffer. Swirl or mix gently and allow to sit for 10 minutes to ensure complete reconstitution. Use control within 1 hour of reconstitution.
   Ranges: IgG1 (1.7 – 2.2 µg/mL)
   IgG2 (0.7 - 1.0 µg/mL)
   IgG3 (0.18 – 0.25 µg/mL)
   IgG4 (0.1 - 0.17 µg/mL)
3. **Standard:** **Human IgG Subclass Standard (Part # 50287HK Lot #: 1694090)**
   Form: Lyophilized, 2 vials. Contains 0.1% sodium azide.
   Storage: Store at 2 to 8ºC.
   Reconstitution: Reconstitute each lyophilized standard vial 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 the Diluent Buffer. When reconstituted in 1.0 mL, the concentrations of the standard are 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 Lot #:** 1694088**)**
   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 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.
5. **Chromogen:** **TMB Solution (Part # SB01 Lot #: 73920539A)**
   Form: 1 vial x 25 mL
   Stop Solution: **Stop Solution (Part # SS03)**
   Form: 1 vial x 25 mL
6. **Diluent:** **Diluent Buffer (Part # 50289HK Lot #: 1699841A)**
   Form: 1 vial x 135 mL
7. **Wash Buffer:** **Wash Buffer Concentrate (25X) (Part # WB01)**
   Form: 100 mL bottle
   Reconstitution: Dilute 1 volume of the 25x wash buffer concentrate with 24 volumes of deionized water (ie. 100 mL may be diluted up to 2.5 liters).
8. **Plate:** **IgG Antibody-Coated Wells, 12 x 8 Well Strips - 2 Plates (Part # 40150 Lot #: 1694089)**

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 appropriate human subclass specific antibody (for example, *MAb Anti-Human IgG1*) to each well in the strip.
3. For the zero wells, add 50 µL of the *Diluent Buffer*. Then, 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 1:2500 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 to 15 seconds, then remove excess by inverting the 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 and in the dark for 10 min**.
8. Quickly add 100 µL of *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 Solution*. Calculate results using a log-log or 4-parameter curve fit.

Explanation of symbols

Symbol	Description	Symbol	Description
	Manufacturer		Catalog number
	Use by		Temperature limitation
	Consult instructions for use		Caution, consult accompanying documents
	Batch code		