Human IgG Subclass Profile ELISA Kit

Cat. No.: DEIA9771
Pkg. Size: 96T

Intended use

The Human IgG Subclass Profile ELISA Kit is intended to allow investigators to quantitate human IgG subclass levels in serum by microtiter plate-based ELISA.

General Description

Five different human immunoglobulin classes have been recognized (IgG, IgA, IgD, IgE and IgM). Of these, IgG constitutes about 70% of the total. The IgG class itself can be divided into 4 unique subclasses (IgG1, IgG2, IgG3, and IgG4). There are many published reports which associate numerous disease states with abnormal levels of one or more IgG subclass.

Principle Of The Test

The 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 an IUIS/WHO study. The captured human IgG is then labelled 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. Human Serum Control, lyophilized, 1 ml
2A. Human IgG Subclass Standard 2A, lyophilized, 1 ml
2B. Human IgG Subclass Standard 2B, lyophilized, 1 ml
2C. Human IgG Subclass Standard 2C, lyophilized, 1 ml
2D. Human IgG Subclass Standard 2D, lyophilized, 1 ml
2E. Human IgG Subclass Standard 2E, lyophilized, 1 ml
2F. Human IgG Subclass Standard 2F, lyophilized, 1 ml
3A. MAb Anti-Human IgG1, 2.5 ml
3B. MAb Anti-Human IgG2, 2.5 ml
3C. MAb Anti-Human IgG3, 2.5 ml
3D. MAb Anti-Human IgG4, 2.5 ml
4. Peroxidase Anti-Human IgG (50x Conc.), 0.5 ml
5A. TMB Single Solution, 2x 11 ml
5B. Stopping Reagent, 11 ml
6. Conjugate/Sample Diluent, 135 ml
7A. Wash Buffer Powder, 1 bag (1 Liter)
7B. 50% Tween-20, 1 ml
8. Microwell Strip (8 wells), 24 strips
9. Strip Holder, 2 each
10. Graph Paper, 1 each
11. Kit Manual, 1 each
Materials Required But Not Supplied

1. Timer
2. Distilled water
3. Multichannel pipette for microtiter plates
4. Pipettes (20-1000 µl) with disposable tips.
5. Container for wash/diluent buffer
6. Microtiter plate reader

Specimen Collection And Handling

Blood samples should be collected by venipuncture. Allow to clot naturally. Undiluted samples may be stored at 2-8°C for up to 72 hours, or at -20°C for longer periods. Avoid repeated freezing and thawing.

Plate Preparation

Microwell strip setup example
(Suggested layout if samples are to be tested in duplicate for all 4 sub classes.)
Figure 1.

![Microwell strip setup example](image1)

Figure 2.
Reagent Preparation

ASSAY INCUBATIONS
Sample/Standards/Control, 30 min, Room Temperature
HRP-Anti-Human IgG Antibody, 30 min, Room Temperature
Ready-To-Use TMB Substrate, 10 min, Room Temperature

1. Prior to use
Allow the kit to warm to room temperature.

2. Working Wash Buffer
Prepare 1 liter of working strength wash buffer by dissolving the wash buffer powder into 1 liter of distilled water. Complete the solution by mixing in 1 ml of 50% Tween-20.

3. Diluted Conjugate Solution
Prepared by diluting concentrated peroxidase-anti-human IgG in conjugate diluent at a ratio of 1:50. For example, add 0.22 ml of conjugate to 11 ml of diluent for each plate (96 wells). Do not prepare more diluted conjugate solution than is needed. For a 96 well plate, 11 ml should be enough. Discard unused portion.

4. Human IgG Subclass Standard/Control
Reconstitute each lyophilized standard and control with 1 ml of deionized water. Vortex or gently agitate to dissolve completely prior to use.

5. Sample Dilutions
The suggested dilution for the patient sample is 1:2500. The investigator, however, may want to find the optimum range for his own sample. Start with an initial dilution of 1:50 by adding 10 μl or 20 μl of samples to 490μl or 980μl of sample diluent respectively. Proceed with another 1:50 dilution by adding 10 μl or 20 μl of the diluted samples to 490μl or 980μl of diluent respectively. The net result is a 1:2500 dilution. Follow each dilution with vortexing or mixing.

Figure 3.
6. Sample Blanks
Rheumatoid factors and other components in human serum can bind nonspecifically to the capture antibody and/or plate to give high false readings. Therefore, blanks which contain only human serum samples and sample diluent will serve as a check for nonspecific binding.

7. Plan Sheet
The six human IgG subclass standands, human serum control, human serum samples, and sample blanks should all be run in duplicate. Prepare a plan sheet for the arrangement of the samples on the plate.

Assay Steps

1. Plan Sheet - Secure desired number of microwells in the microwell holder according to the arrangement of the plan sheet.
2. Plate Washing - Dispense 200 µl of wash buffer into each well and remove by flicking into the sink and slap dry on a paper towel.
3. Patient Sample/Control/Standards/Subclass Specific Antibody Dispensing - Dispense 50 µl of diluted serum samples, ready-to-use standards, and the ready-to-use control to their respective wells according to the plan sheet. Then, add 50 µl of the appropriate human subclass specific antibody to each well except for blanks. For the blank wells, dispense 50 µl of diluted serum samples. Then, add 50 µl of the sample diluent.
4. Incubation - Incubate the plate at room temperature for 30 min.
5. Plate Washing - Remove contents by flicking into the sink. Repeat Step 2, three times.
6. Conjugate dispensing - Dispense 100 µl of diluted conjugate solution into each well.
7. Incubation - Incubate the plate at room temperature for 30 min.
8. Plate Washing - Remove contents by flicking into the sink. Repeat Step 2, three times.
9. Substrate dispensing - Dispense 100 µl of the ready-to-use TMB substrate into each well.
10. Incubation - Incubate the plate at room temperature for 10 min.
11. Stopping Reagent - Quickly dispense 50 µl of stopping reagent into each well and shake for a few seconds. A dramatic color change from blue to yellow should occur.
12. Absorbance Reading - Readings can be made immediately at a wavelength of 450 nm and a reference wavelength of 550 nm (latter one is not required). The OD’s should remain stable for 1 hour.
13. Plotting and Determination of IgG Subclass Conc.-It is recommended that standard curves be prepared by graphing Absorbance vs. Log concentration for each IgG subclass. Concentration of the standards are listed in the STANDARD VALUES section. However, other data reduction methods may also be used. The IgG subclass concentration of each sample and control can then be calculated from the standard curve using the respective OD of each sample or control.

**Note:**

STANDARD CURVE- A six point standard curve is used to determine the concentration of the human IgG subclass. Each standard is provided lyophilized and is to be reconstituted with 1 ml of deionized water. No further dilution is necessary. Blanks are not necessary.

SAMPLES - One dilution is used for each sample. The sample is pipetted in duplicate into its corresponding wells. Single wells are used for blanks.

CONTROL - The prediluted control is treated exactly the same as a sample. The control is pipetted in duplicate into its corresponding wells. Blanks, however, are not necessary.

BLANKS - Single well blanks are only necessary for the samples. Unlike the other wells, these do not contain MAb Anti-Human IgG subclass and are used as qualitative checks.

**Quality Control**

Each laboratory should run controls and sample blanks in every assay to assure validity of the results. Acceptable control ranges should be established by each laboratory. High sample blanks may indicate the presence of rheumatoid factors or other agents in the sample which can bind nonspecifically.

**Calculation**

**SUBCLASS CONCENTRATIONS**

1. From the appropriate Human Subclass Standard Graph, read off the concentration (x axis) corresponding to the sample OD (y axis).

2. Calculate the sample subclass concentration from the following formula: IgG Subclass = (1/Dilution Factor) x Concentration (read from x axis)

**Examples of Calculations:**

The following examples use the typical standard curves shown in the right column.

Calculation of IgG1

\[ \text{X} = 4.0 \, \mu g/ml \quad \text{Y} = 0.77 \, \text{OD units} \]

\[ \text{IgG1} = 2500 \times 4.0 = 10,000 \, \mu g/ml \]

Calculation of IgG2

\[ \text{X} = 1.7 \, \mu g/ml \quad \text{Y} = 0.60 \, \text{OD units} \]

\[ \text{IgG2} = 2500 \times 1.7 = 4,250 \, \mu g/ml \]

Calculation of IgG3

\[ \text{X} = 0.2 \, \mu g/ml \quad \text{Y} = 0.65 \, \text{OD units} \]

\[ \text{IgG3} = 2500 \times 0.2 = 500 \, \mu g/ml \]

Calculation of IgG4

\[ \text{X} = 0.06 \, \mu g/ml \quad \text{Y} = 0.64 \, \text{OD units} \]

\[ \text{IgG4} = 2500 \times 0.06 = 150 \, \mu g/ml \]

**Typical Standard Curve**

**STANDARD VALUES**

Table 1.
STANDARD GRAPHS FOR HUMAN IgG SUBCLASSES

Typical Standard Curve for Human IgG1
Figure 4.

Typical Standard Curve for Human IgG2
Figure 5.
Typical Standard Curve for Human IgG3
Figure 6.

Typical Standard Curve for Human IgG4
Figure 7.
REFERENCES