Human IgG ELISA
Life Diagnostics, Inc., Catalog Number: IGG-20

INTRODUCTION

Pharmacokinetic (PK) characterization of human IgG monoclonal antibodies requires measurement of human IgG in serum of species used in preclinical research. This kit allows measurement of human IgG1-4 subclasses[1] in serum of mice, rats, dogs, and monkeys[2].

PRINCIPLE OF THE ASSAY

The human IgG ELISA uses affinity purified goat anti-human IgG for solid phase (microtiter wells) immobilization and horseradish peroxidase (HRP) conjugated anti-human IgG for detection. Test samples are diluted and incubated in the microtiter wells for 45 minutes alongside human IgG standards. The microtiter wells are subsequently washed and HRP conjugate is added and incubated for 45 minutes. IgG molecules are thus sandwiched between the immobilization and detection antibodies. The wells are then washed to remove unbound HRP-conjugate and TMB reagent is added and incubated for 20 minutes. This results in the development of a blue color. Color development is stopped by the addition of Stop solution, changing the color to yellow. Absorbance is measured at 450 nm. The concentration of IgG is proportional to the absorbance of the test sample and is derived from a standard curve.

MATERIALS AND COMPONENTS

Materials provided with the kit:

- Anti-human IgG coated 96-well plate (12 strips of 8 wells)
- HRP Conjugate, 11 ml
- Human IgG stock
- 20x Wash Solution: TBS50-20, 50 ml
- Diluent: YD50-1, 50 ml
- TMB reagent: TMB11-1 11 ml
- Stop solution: SS11-1, 11 ml

Materials required but not provided:

- Precision pipettes and tips
- Distilled or deionized water
- Polypropylene or glass tubes
- Vortex mixer
- Absorbent paper or paper towels
- Micro-Plate incubator/shaker mixing speed of ≈150 rpm
- Plate washer
- Plate reader capable of measuring at 450nm
- Curve-fitting software

STORAGE

The test kit will remain stable for six months from the date of purchase provided that the components are stored at 4°C. The microtiter plate should be kept in a sealed bag with desiccant to minimize exposure to damp air.

GENERAL INSTRUCTIONS

1. Please read and understand the instructions thoroughly before using the kit.
2. All reagents should be allowed to reach room temperature (25°C) before use.
3. Optimal results are achieved if, at each step, reagents are pipetted into the wells of the microtiter plate within 5 minutes.

WASH SOLUTION PREPARATION

The wash solution is provided as a 20x stock. Prior to use, dilute the contents of the bottle (50 ml) with 950 ml of distilled or deionized water.

STANDARD PREPARATION

1. The human IgG stock is provided in lyophilized form. Reconstitute as detailed on the vial label ***(the reconstituted standard is stable at 4°C for one day but should be aliquoted and frozen at -20°C after reconstitution if future use is intended)***.
2. Label 8 polypropylene or glass tubes as 50, 25, 12.5, 6.25, 3.13, 1.56, 0.78 and 0 ng/ml.
3. Into the tube labeled 50 ng/ml, pipette the volume of diluent detailed on the IgG stock vial label. Then add the indicated volume of IgG stock and mix gently. This provides the 50 ng/ml standard.
4. Dispense 250 µl of diluent into the tubes labeled, 25, 12.5, 6.25, 3.13, 1.56, 0.78 and 0 ng/ml.
5. Prepare a 25 ng/ml standard by diluting and mixing 250 µl of the 50 ng/ml standard with 250 µl of diluent in the tube labeled 25 ng/ml.
6. Similarly prepare the 12.5, 6.25, 3.13, 1.56 and 0.78 ng/ml standards by two-fold serial dilution.

SAMPLE PREPARATION

Because the concentrations of human IgG in PK studies depend on the study format it is not possible for us to recommend an optimal dilution factor. Optimal dilutions must be determined by the end user. However, all serum or plasma samples must be diluted at least 10-fold to avoid matrix effects. Only use the diluent provided with the kit. Use diluted samples within 30 minutes.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense 100 µl of standards and diluted samples into the wells (we recommend that standards and samples be tested in duplicate).
3. Incubate in a plate shaker/incubator at 150 rpm at 25°C for 45 minutes.
4. Aspirate the contents of the microtiter wells and wash the wells 5 times with 1x wash solution using a plate washer (400 µl/well). The entire wash procedure should be performed as quickly as possible.
5. Strike the wells sharply onto absorbent paper or paper towels to remove all residual wash buffer.
6. Add 100 µl of HRP conjugate into each well.
7. Incubate in a plate shaker/incubator at 150 rpm at 25°C for 45 minutes.
8. Wash as detailed in 4 to 5 above.
9. Dispense 100 µl of TMB Reagent into each well.

[1] The relative reactivities of IgG(total) : IgG1 : IgG2: IgG3 : IgG4 were determined to be 1.05 : 1.0 : 0.25 : 0.78 : 0.30.

[2] The kit was validated using cynomolgus monkey serum.

Life Diagnostics, Inc., P.O. Box 5205, West Chester, PA 19380
610-431-7707 – 610-431-7818 (Fax)
info@lifediagnostics.com – www.lifediagnostics.com

10. Incubate in a plate shaker/incubator at 150 rpm at 25°C for 20 minutes.
11. Stop the reaction by adding 100 µl of Stop solution to each well.
12. Gently mix. *It is important to make sure that all the blue color changes to yellow.*
13. Read the absorbance at 450 nm with a microtiter plate reader within 5 minutes.

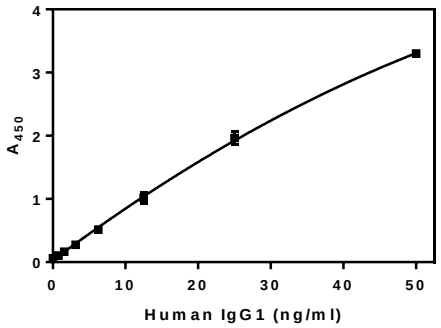
CALCULATION OF RESULTS

1. Using graphing software, construct a standard curve by plotting A_{450} values for the standards versus IgG concentration.
2. Fit the data to a single site, total and non-specific binding model.
3. Derive the concentrations of the samples.
4. Multiply the derived concentrations by the respective dilution factors to determine the actual concentration of IgG in the sample.
5. If the A_{450} values of samples fall outside the standard curve, samples should be diluted appropriately and re-tested.

TYPICAL STANDARD CURVE

A typical standard curve with A_{450} values on the Y-axis against IgG concentrations on the X-axis is shown below. The curve is for illustration only and should not be used to calculate unknowns.

IgG (ng/ml)	Absorbance (450 nm)
50	3.300
25	1.866
12.5	0.953
6.25	0.482
3.13	0.258
1.56	0.156
0.78	0.102
0	0.071



LIMITATIONS OF THE PROCEDURE

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of and in accordance with the instructions detailed above.
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

ASSAY PERFORMANCE

Serum from swiss-webster mice, sprague dawley rats, mongrel dogs, and cynomolgus monkeys was spiked with 100 or 1000 ng/ml of human IgG1. Samples were tested at dilutions of 10, 20, 40… to 1280-fold. Recovery values were 100%. No significant human IgG reactivity was found in unspiked serum from the above species.

Rev 032125

For technical assistance please email us at
techsupport@lifediagnostics.com