Catalog # HUIGGKT

Human IgG Antigen Assay

Strip well format. Reagents for up to 96 tests.

For Research Use Only.

INTENDED USE

This human immunoglobulin G antigen assay is intended for the quantitative determination of total human IgG antigen in serum, plasma, hybridoma cell supernatants, ascites or other biological fluids. The assay does not distinguish IgG subclasses.

BACKGROUND

IgG is the most abundant immunoglobulin in serum and is predominately involved in the secondary immune response. The IgG subclasses are designated 1, 2, 3 and 4 based on their relative prevalence in human serum.

ASSAY PRINCIPLE

Human IgG will bind to the affinity purified capture antibody coated on the microtiter plate. After appropriate washing steps, horseradish peroxidase labeled polyclonal anti-human IgG antibody binds to the captured protein. Excess antibody is washed away and TMB substrate is used for color development at 450nm. A standard calibration curve is prepared along with the samples to be measured using dilutions of human IgG. Color development is proportional to the concentration of IgG in the samples.

STANDARD CALIBRATION

Human IgG standard provided is calibrated against the WHO International Standard for Immunoglobulins G, A and M, Human Serum distributed by NIBSC (67/086), South Mimms, Potters Bar, Hertfordshire, UK.

Lot 1111L: 500 ng = 0.00715 U

REAGENTS PROVIDED

- **96-well microtiter strip plate:**
  8X12 removable well strips containing affinity purified anti-human IgG antibody dried and blocked on the surface
- **10X Wash Buffer:**
  1 bottle of 50ml; bring to 1X using DI water
- **Human IgG antigen standard:**
  1 vial of lyophilized standard
- **Peroxidase anti-human IgG antibody:**
  1 vial of lyophilized HRP labeled antibody
- **TMB substrate solution:**
  1 bottle of 10ml solution

STORAGE AND STABILITY

All kit components must be stored at 4°C. Store unopened plate and any unused microtiter strips in the pouch with desiccant. Reconstituted standards and primary may be stored at -70°C for later use. **DO NOT** freeze/thaw the standards and primary antibody more than once. All other unused kit components must be stored at 4°C. Kit should be used no later than the expiration date.

REAGENTS AND EQUIPMENT REQUIRED

- 1-channel pipettes covering 0-10µl and 200-1000µl
- 12-channel pipette covering 30-300µl
- Paper towels or kimwipes
- 50ml tubes, 1.5ml centrifuge tubes
- 1N H_2SO_4
- DI water
- Magnetic stirrer and stir-bars
- Plastic containers with lids
- Microtiter plate spectrophotometer operable at 450nm
- Microtiter plate shaker with uniform horizontally circular movement up to 300rpm.

WARNINGS

Warning – Avoid skin and eye contact when using TMB One substrate solution since it may be irritating to eyes, skin, and respiratory system. Wear safety goggles and gloves.

PRECAUTIONS

- **DO NOT** mix any reagents or components of this kit with any reagents or components of any other kit. This kit is designed to work properly as provided.
- **DO NOT** pipette reagents by mouth.
- Always pour substrate out of the bottle into a clean test tube. **DO NOT** pipette out of the bottle as you could contaminate the substrate.
- Keep plate covered except when adding reagents, washing, or reading.
- **DO NOT** smoke, drink, or eat in areas where specimens or reagents are being handled.

PREPARATION OF REAGENTS

- **TBS buffer:** 0.1M Tris 0.15M NaCl pH 7.4
- **Blocking buffer (BB):** 3% BSA in TBS
- **Wash buffer concentrate:** The wash buffer supplied in a 10X concentrate and must be diluted 1:10 with deionized water for use with the kit.

SPECIMEN COLLECTION

The assay measures total human IgG in the 1-500 ng/ml range. Samples giving human IgG levels above 500ng/ml should be diluted in Blocking buffer before use. A 1:500,000 dilution for serum, serially generated by 1:100, 1:100, and 1:50 dilutions, is suggested for best results.

ASSAY PROCEDURE

Perform assay at room temperature. Vigorously shake plate (300rpm) at each step of the assay.

Preparation of Standard:

Reconstitute standard vial with 1 ml of BSA blocking buffer to give a 500ng/ml solution.

Dilution table for preparation of human IgG standards:

IgG concentration (ng/ml)	Dilutions
500	Straight from the vial
200	600µl (BB) + 400µl (500ng/ml)
100	500µl (BB) + 500µl (200ng/ml)
50	500µl (BB) + 500µl (100ng/ml)
20	600µl (BB) + 400µl (50ng/ml)
10	500µl (BB) + 500µl (20ng/ml)
5	500µl (BB) + 500µl (10ng/ml)
2	600µl (BB) + 400µl (5ng/ml)
1	500µl (BB) + 500µl (2ng/ml)
0.5	500µl (BB) + 500µl (1ng/ml)
0	500µl (BB) Zero point to determine background

NOTE: DILUTIONS FOR THE STANDARD CURVE AND ZERO STANDARD MUST BE MADE AND APPLIED TO THE PLATE IMMEDIATELY.

Standard and Unknown Addition:
Remove microtiter plate from bag. Add 100µl standards in duplicate and unknowns to wells. Carefully record position of standards and unknowns. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

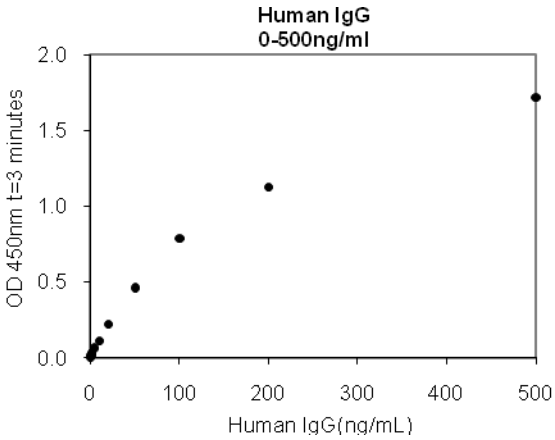
Peroxidase Antibody Addition:
Reconstitute peroxidase conjugated antibody by adding 10ml BSA blocking buffer to vial. Agitate gently to completely dissolve contents. Add 100µl to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

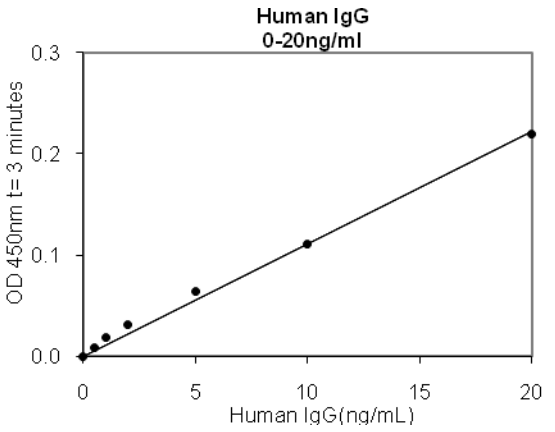
Substrate Incubation:
Add 100µl of TMB substrate solution to all wells and shake plate at 300rpm for 2-10 minutes. Quench the reaction with the addition of 50µl of 1N H_2SO_4 and read final absorbance values at 450nm.
NOTE: Time for substrate development is dependent on needs of researcher.

Measurement:
Set the absorbance at 450nm in a microtiter plate spectrophotometer. Measure the absorbance in all wells at 450nm. Subtract zero point from all standards and unknowns to determine corrected absorbance (A_{450}).

Assay Calibration:
Plot A_{450} against the amount of human IgG in the standards. Fit a straight line through the points using a linear fit procedure. The amount of total human IgG in the unknowns can be determined from this curve.

A typical standard curve.
(EXAMPLE ONLY, DO NOT USE)





EXPECTED VALUES

The concentration of IgG in normal human serum ranges from 5 to 12 mg/ml.

DISCLAIMER

This information is believed to be correct but does not claim to be all-inclusive and shall be used only as a guide. The supplier of this kit shall not be held liable for any damage resulting from handling or from contact with the above product.

Example of Kit Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	0.5ng/ml	1ng/ml	2ng/ml	5ng/ml	10ng/ml	20ng/ml	50ng/ml	100ng/ml	200ng/ml	500ng/ml	
B	0	0.5ng/ml	1ng/ml	2ng/ml	5ng/ml	10ng/ml	20ng/ml	50ng/ml	100ng/ml	200ng/ml	500ng/ml	
C												
D												
E												
F												
G												
H												

96 Well Plate
Standards: 22 wells
Samples: 74 wells