

INTENDED USE

This human immunoglobulin G subclass 4 (IgG4) antigen assay is intended for the quantitative determination of total human IgG4 antigen in serum, plasma, hybridoma cell supernatants, ascites or other biological fluids. The assay does not detect immunoglobulins of other classes or subclasses. **For research use only.**

BACKGROUND

IgG4 is the least abundant IgG subclass in serum and is frequently induced by repeated allergen exposure [1]. IgG4 does not have allotype variation and does not interact with complement component C1q [2]. Clinically elevated serum IgG4 levels occur in a variety of conditions including the multi-organ fibro-inflammatory IgG4-related disease [3].

ASSAY PRINCIPLE

Human IgG4 in samples will bind to the biotinylated capture antibody which is bound to the avidin on the 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 IgG4. Color development is proportional to the concentration of IgG4 in the samples.

REAGENTS PROVIDED

- **96-well avidin coated microtiter strip plate** (removable wells 8x12) containing avidin, blocked and dried.
- **10X Wash buffer:** 1 bottle of 50ml
- **Biotinylated anti-human IgG primary antibody:** 1 vial lyophilized polyclonal antibody
- **Human IgG4 standard:** 1 vial lyophilized standard
- **Anti-human horseradish peroxidase secondary antibody:** 1 vial concentrated HRP labeled antibody
- **TMB substrate solution:** 1 bottle of 10ml solution

STORAGE AND STABILITY

Store all kit components at 4°C upon arrival. Return any unused microplate strips to the plate pouch with desiccant. Reconstituted standard and primary may be stored at -80°C for later use. Do not freeze-thaw the standard and primary antibody more than once. Store all other unused kit components at 4°C. This kit should not be used beyond the expiration date.

OTHER REAGENTS AND SUPPLIES REQUIRED

- Microtiter plate shaker capable of 300 rpm uniform horizontally circular movement
- Manifold dispenser/aspirator or automated microplate washer
- Microplate reader capable of measuring absorbance at 450 nm
- Pipettes and Pipette tips
- Deionized or distilled water
- Polypropylene tubes for dilution of standard
- Paper towels or laboratory wipes
- 1N H₂SO₄ or 1N HCl
- Bovine Serum Albumin Fraction V (BSA)
- Tris(hydroxymethyl)aminomethane (Tris)
- Sodium Chloride (NaCl)

PRECAUTIONS

- **FOR LABORATORY RESEARCH USE ONLY. NOT FOR DIAGNOSTIC USE.**
- 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.
- Always pour peroxidase substrate out of the bottle into a clean test tube. Do not pipette out of the bottle as contamination could result.
- Keep plate covered except when adding reagents, washing, or reading.
- **DO NOT** pipette reagents by mouth and avoid contact of reagents and specimens with skin.
- **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 (w/v) in TBS
- 1X Wash buffer:** Dilute 50ml of 10X wash buffer concentrate with 450ml of deionized water

SAMPLE COLLECTION

Collect plasma using EDTA or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000xg within 30 minutes of collection. Assay immediately or aliquot and store at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

ASSAY PROCEDURE

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

Primary Antibody Addition

Remove microtiter plate from bag, reconstitute primary antibody by adding 10ml of blocking buffer directly to the vial and agitate gently to completely dissolve contents. Add 100 μl of this dilution to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300 μl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

Preparation of Standard

Reconstitute standard by adding 1ml of blocking buffer directly to the vial and agitate gently to completely dissolve contents. This will result in a 1000ng/ml standard solution.

Dilution table for preparation of human IgG4 standard:

IgG4 concentration (ng/ml)	Dilutions
500	500 μl (BB) + 500 μl (from vial)
200	600 μl (BB) + 400 μl (500ng/ml)
100	500 μl (BB) + 500 μl (200ng/ml)
50	500 μl (BB) + 500 μl (100ng/ml)
20	600 μl (BB) + 400 μl (50ng/ml)
10	500 μl (BB) + 500 μl (20ng/ml)
5	500 μl (BB) + 500 μl (10ng/ml)
2	600 μl (BB) + 400 μl (5ng/ml)
1	500 μl (BB) + 500 μl (2ng/ml)
0.5	500 μl (BB) + 500 μl (1ng/ml)
0	500 μl (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

Add 100 μl IgG4 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 300 μl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

NOTE: The assay measures total human IgG4 antigen in the 0.5-500 ng/ml range. If the unknown is thought to have IgG4 levels greater than 500ng/ml, dilutions may be made in blocking buffer before use. A 1:20,000-1:100,000 dilution for normal human plasma is suggested for best results.

Secondary Antibody Addition

Briefly centrifuge vial before opening. Dilute 1 μl of conjugated secondary antibody in 10ml of blocking buffer and add 100 μl to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300 μl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

Substrate Incubation

Add 100 μl TMB substrate to all wells and shake plate for 2-10 minutes. Substrate will change from colorless to different strengths of blue. Quench reaction by adding 50 μl of 1N H_2SO_4 or HCl stop solution to all wells when samples are visually in the same range as the standards. Add stop solution to wells in the same order as substrate upon which color will change from blue to yellow. Mix thoroughly by gently shaking the plate.

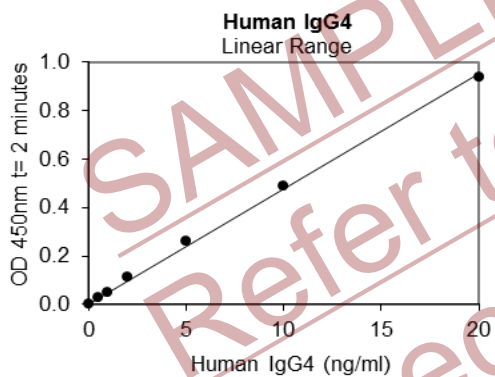
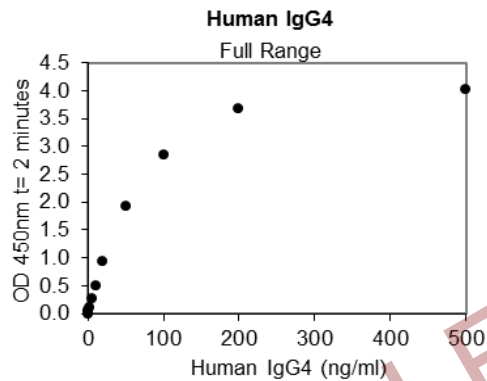
Measurement

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}).

Calculation of Results

Plot A_{450} against the amount of IgG in the standards. Fit a straight line through the linear points of the standard curve using a linear fit procedure if unknowns appear on the linear portion of the standard curve. Alternatively, create a standard curve by analyzing the data using a software program capable of generating a four parameter logistic (4PL) curve fit. The amount of IgG4 in the unknowns can be determined from this curve. If samples have been diluted, the calculated concentration must be multiplied by the dilution factor.

A typical standard curve (EXAMPLE ONLY):



EXPECTED VALUES

The mean value of IgG4 in serum of healthy donors was found to be 1.04 mg/ml [4].

PERFORMANCE CHARACTERISTICS

Sensitivity: The minimum detectable dose (MDD) was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates (range OD₄₅₀: 0.048-0.061) and calculating the corresponding concentration. The MDD was 0.25 ng/mL.

Intra-assay Precision: These studies are currently in progress. Please contact us for more information.

Inter-assay Precision: These studies are currently in progress. Please contact us for more information.

Recovery: These studies are currently in progress. Please contact us for more information.

Linearity: These studies are currently in progress. Please contact us for more information.

Sample Values: Samples were evaluated for the presence of the antigen at varying dilutions.

Sample Type	Dilution	Mean (mg/mL)
Citrate Plasma	1:40,000	0.96
	1:80,000	0.95
Serum	1:40,000	0.85
	1:80,000	0.84

REFERENCES

1. Vidarsson G, *et al.*: Front Immunol. 2014, 5:520.
2. Irani V, *et al.*: Mol Immunol. 2015, 67:171-82.
3. Wallace ZS, *et al.*: Arthritis Rheumatol. 2015, 67:2466-75.
4. Yu KH, *et al.*: Medicine (Baltimore). 2015, 94:e1707.

Example of ELISA Plate Layout

96 Well Plate: 22 Standard wells, 74 Sample wells

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

SAMPLE INSERT
Refer to kit box for
lot specific instructions