ABSbio™ Human IgG Fc ELISA Kit (SE018-HUFC)

Human Fc quantitative Assay

INTRODUCTION

ABSbio™ Human IgG Fc ELISA kit is used for the quantification of human IgG Fc and Fc fusion protein in serum, plasma, cell supernatant and other matrixes. This assay is specific for human IgG Fc, and does not cross react with mouse IgG Fc. The kit uses a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) to analyze the level of human Fc in samples. A Monoclonal Antibody specific for Human IgG Fc has been pre-coated onto a 96-well microplate. Standards and unknown samples were loaded into the wells and incubated. After washing away any unbound substances, an enzyme-linked anti-human antibody specific for IgG Fc is added to the wells and incubated. Following a washing to remove any unbound antibody-enzyme reagent. Tetramethylbenzidine (TMB) is added to the microplate wells. TMB reacts with peroxide in the presence of HRP to form a colorimetric signal proportional to the amount of IgG Fc bound by the capture antibody. Color development is stopped by the addition of acid to the microplate wells. The Stop Solution changes the color from blue to yellow and the intensity of the color optical density (OD) is measured at 450 nm using a spectrophotometer. The minimum detectable dose of this kit typically less than 0.02 ng/mL, detection range is 0.01-2 ng/mL.

KIT COMPONENTS

Human Fc Standards:	1 vial	Assay Diluent:	50 mL	TMB Solution:	12 mL	Plate sealer:	2
Detection Antibody :	60 µL	Wash Solution (10x):	50 mL	Stop Solution:	12 mL	Anti-Human Fc antibody coated plate:	1

Storage and Handling: Shipping on ice. Store kit at 2-8 ℃. Shelf Life: 3 months after receipt. Bring all reagents and materials to room temperature before assay. Remove any unused antibody coated strips from the micro-plate, return them to the foil pouch and re-seal.

OTHER MATERIALS REQUIRED, BUT NOT PROVIDED

1. Pipettes and pipette tips. 2. 96-well plate or manual strip washer. 3. Buffer and reagent reservoirs. 4. Paper towels or absorbent paper. 5. Plate reader capable of reading absorbency at 450 nm. 6. Distilled water or deionized water. 7. Micro-plate shaker capable of 600 rpm.

PREPARATION OF REAGENTS

- **Wash Solution**: 10x dilute Wash Solution with dH_2O to prepare 1x Wash Solution.
- **Human Fc Protein Standard:** The vial contains 10 µl of the standard sufficient for a 96-well plate. Add 2 µl of the standard into 998 µl of Assay Diluent to make the high standard concentration of 2 ng /mL. A seven point standard curve is generated using 2-fold serial dilutions in Assay Diluent, vortex briefly for each of dilution step.
- **Detection Ab:** Immediately before use, add 50 µl of the antibody into 11 mL of Assay Diluent for one plate (for partial plate assay, adjust the volumes accordingly).
- **Sample:** Sample dilution with Assay diluent may need to be optimized by customer.

PROCEDURE GUIDELINE

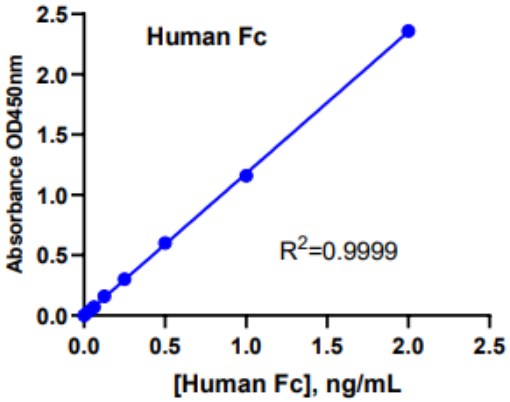
1. It is recommended that all standards and samples should be run in duplicate. Set standard wells, testing sample and blank wells on the assay plate/strip. Transfer diluted standard 100 µl to standard wells, diluted sample 100 µl to sample wells, Assay diluent 100 µl only to blank wells.

	1	2	3	4	5	6	7	8	9	10	11	12
A	2000 pg/mL standard	2000 pg/mL standard	sample1 (1:10)	sample1 (1:10)								
B	1000 pg/mL	1000 pg/mL	sample1 (1:100)	sample1 (1:100)								
C	500 pg/mL	500 pg/mL	sample1 (1:1000)	sample1 (1:1000)								
D	250 pg/mL	250 pg/mL	sample1 (1:10000)	sample1 (1:10000)								
E	125 pg/mL	125 pg/mL	Sample2 (1:10)	Sample2 (1:10)								
F	62.5 pg/mL	62.5 pg/mL	s sample2 (1:100)	s sample2 (1:100)								
G	31.25 pg/mL	31.25 pg/mL	Sample2 (1:1000)	Sample2 (1:1000)								
H	blank	blank	Sample2 (1:10000)	Sample2 (1:10000)								

2. Cover the plate with plate sealer and incubate the plate at room temperature for 2 hrs or at 37 ℃ for 1 hr, shaking the plate on a micro-plate shaker.
3. Decant as much liquid as possible, fill the wells with 300 µl wash solution, shaking plate for 5 min, decant the wash solution and remove residual liquid with absorbent paper. Repeat wash four times.
4. Add 100 µl of 1x Detection antibody solution per well. Cover the plate with plate sealer and incubate the plate at room temperature for 1h, shaking the plate on a micro-plate shaker.
5. Wash 5 times as outlined in step 3.
6. Add 100 µl of TMB Solution to each well and incubate at room temperature for 20~30 minutes protect from light, or keep close monitoring on the developing process until desired developing blue color observed. **Note: please be aware that the color may develop more quickly or more slowly that the recommended incubation time depending on the localized room temperature.**

7. Add 100 µl of Stop Solution to each well to stop the reaction (the blue color change to yellow), gently tap the plate frame for a few seconds to ensure thorough mixing.
8. Read absorbance of the plate on a microplate reader at 450 nm within 15 min.
9. Average the duplicate readings for each standard and samples, subtract the average zero (blank) standard optical density. Construct standard curve (plotting the mean OD450 for each standard on the X-axis against concentration on the Y-axis, draw a best-fit curve through the points) and calculate linear regression equation, then use corrected sample OD values and regression equation to calculate the corresponding sample concentration. It should be remembered that the sample has been diluted and its actual concentration should be justified by dilution factor (the measurement and calculation can be accomplished by software like SoftMax).
10. If molecular weight of sample differs from Fc standard (MW 50kD) apply the following equation to the reading concentration to obtain the actual concentration**=[MW Sample]/[MW Fc protein] x Sample reading (ng/mL)**

Typical Standard Curve



SUMMARY OF ASSAY PROCEDURE

Add 100 µl of standard or sample to each well.

Incubate at room temperature for 1 hours.

Wash each well 4 times.

Add 100 µl of 1× Detection antibody solution per well.

Incubate at RT for 1 hour. Wash 5 times.

Add 100 µl of TMB solution to each well.

Incubate at room temperature for 20-30 minutes.

Add 100 µl of Stop solution to each well.

Measure absorbance of each well at 450 nm.

Calculation

Always run your own standard curves for calculation of results.

ASSAY CHARACTERISTICS

A. Sensitivity
The lowest human Fc level that can be measured by this assay is 0.01 ng/ml.

B. **Precision**
Intra-assay Precision (Precision within an assay) C.V. < 7.6%.
Inter-assay Precision (Precision between assays) C.V. <8%.

C. **Recovery**
The recovery of the assay was determined by adding various amounts human Fc to a sample. The measured concentration of the spiked sample in the assay was compared to the expected concentration. The average recovery was 96-103 %.

D. **Specificity**
Percent of cross reactivity
Human IgG Fab, IgA, IgM undetectable
Mouse, Rat, Rabbit IgG undetectable

TROUBLE SHOOTING GUIDE

1. To obtain reliable and reproducible results the operator should carefully read this manual and fully understand all aspects of each assay step before attempting to run the assay.
2. Throughout the assay the operator should adhere strictly to the procedures with good laboratory practice.
3. Have all necessary reagents and equipment ready on hand before starting. Once the assay has been started all steps should be completed with precise timing and without interruption.
4. Avoid cross contamination of any reagents or samples to be used in the assay.
5. Make sure that all reagents and samples are added to the bottom of each well.
6. Careful and complete mixing of solutions in the well is critical. Poor assay precision will result from incomplete mixing or cross well contamination due to inappropriate mixing.
7. Remove any air bubble formed in the well after acidification of substrate solution because bubbles interfere with spectrophotometric readings.
8. High absorbance in background or blank wells could be due to 1) cross well contamination by standard solution or sample or 2) inadequate washing of wells with washing solution.