Human IgG ELISA kit

IBT Bioservices cat# 0101-001, lot# 2304002

Instructions for use

1. Purpose:

For the quantitative measurement of human IgG.

2. Reagents supplied:

Reagent supplied	Lot Number	Protein Conc.	Amount	Storage Temperature
DPBS 1X for diluting Capture Antibody	N/A	N/A	13 mL	4°C
Capture Antibody	**03.15.2023**	**1.0 mg/mL**	**30 µL**	short-term **4°C** long-term **-20°C**
Assay Diluent for blocking and for diluting Standard, Samples, Secondary Antibody & Detection Reagent	N/A	N/A	80 mL	4°C
Human IgG Standard	**02.09.2019-B**	**1.0 mg/mL**	**15 µL**	short-term **4°C** long-term **-20°C**
Secondary Antibody	**01.26.2021**	**0.474 mg/mL**	**30 µL**	short-term **4°C** long-term **-20°C**
Detection Reagent	**N/A**	**0.500 mg/mL**	**15 µL**	**4°C**
TMB one-step substrate	N/A	N/A	13 mL	4°C

3. Reagents required but not included in the kit:

- DPBS powder (Corning cat# 55-031-PB) or equivalent, stored at 4°C, for preparing ELISA Wash Buffer. For more information, please refer to page 6.
- TWEEN-20 (Sigma-Aldrich cat# P1379-500 mL) or equivalent, stored at ambient temperature, for preparing ELISA Wash Buffer. For more information, please refer to page 6.
- Deionized water

4. Materials required but not included in the kit:

- MaxiSorp flat-bottom, polystyrene, 96-well plates (Thermo Scientific cat# 439454)
- Polypropylene TiterTubes, maximum volume for each tube = 1 mL (Bio-Rad cat# 223-9391) or equivalent, used to prepare standard and sample dilutions
- Microplate sealing film
- Polypropylene 15 mL and 50 mL conical tubes
- Reagent reservoirs
- Absorbent papers

5. Equipment required but not included in the kit:

- Automatic plate washer (example: BioTek ELx450)
- Plate reader with capability of measuring absorbance at 650 nm (example: Molecular Devices plate reader)
- Software for graphing the Standard as a 4PL curve and for calculating the Unknown Samples from the standard curve (example: Softmax software)
- Single-channel and multi-channel pipettes

6. Assay Procedure:

1. Prepare Capture antibody solution
    - Briefly spin the **Capture Antibody (1.0 mg/mL)** vial and gently mix by pipetting up and down
    - **Dilute 1:500 in DPBS 1X to target 2 µg/mL**
    - **Example: For one full plate, add 24 µL Capture antibody to 12 mL of DPBS 1X**
2. Add 100 µL/well of Capture antibody solution to the MaxiSorp plate. Cover plate using plate sealing film. Incubate covered plate overnight at 4°C.
3. The following day, equilibrate plate and Assay Diluent to ambient temperature for at least 15 min.
4. Empty contents from the plate and wash 3 times (each time 300 µL/well) with Wash Buffer using an automatic plate washer or multi-channel pipette.
5. Add 250 µL/well of Assay Diluent to block non-specific binding. Incubate for at least 1 hour at ambient temperature.
6. During blocking step, prepare dilutions of STANDARD and UNKNOWN SAMPLES in TiterTubes. Each dilution should contain sufficient volume (>200 µL) for duplicate wells.

a. STANDARD

- Briefly spin the **Standard (1.0 mg/mL)** vial and gently mix by pipetting up and down
- First dilution = 1:10 to target 100 µg/mL

Add 10 µL STANDARD at 1.0 mg/mL to 90 µL of Assay Diluent. This is 10 µg/mL INTERMEDIATE.

- **Second dilution = 1:10 to target 10 µg/mL**

Add 50 µL INTERMEDIATE at 100 µg/mL to 450 µL of Assay Diluent. This is the 10 µg/mL starting concentration tested on ELISA plate.

Use a new pipet tip to gently mix by pipetting up and down**.**

- **Serial 1:3.162-fold dilutions**
  - **Transfer 139 µL from the previous dilution to 300 µL Assay Diluent.**
  - Discard pipet tip
  - Use a new pipet tip to gently mix by pipetting up and down. Change pipet tips between each dilution.
  - Repeat for subsequent dilutions

b. UNKNOWN TEST SAMPLES

Note: Mouse and rat serum at dilutions 1:100, 1:1,000 and 1:10,000 did not interfere with the recovery of the Human IgG standard (please see page 9). Other matrices or lower dilutions have not been tested.

- Prepare sample dilutions using Assay Diluent at dilution factors determined by the end-user
- Remember to mix well by pipetting solution up and down a few times and change pipet tips in between dilutions
- The followings are suggestions for sample dilution factors:

Unknown Test Sample	Dilution Factor	
Dilution 1	**1:100**	1:100 dilution Add 5 µL Unknown Sample to 495 µL of Assay Diluent
Dilution 2	**1:1,000**	1:10 of 100 dilution Add 30 µL Dilution 1 to 270 µL of Assay Diluent
Dilution 3	**1:10,000**	1:10 of 1,000 dilution Add 30 µL Dilution 2 to 270 µL of Assay Diluent

7. Empty contents from the plate and wash 3 times (each time 300 µL/well) with Wash Buffer using automatic plate washer or multi-channel pipette.

8. Use multi-channel pipettor to transfer 100 µL/well of STANDARD or UNKNOWN SAMPLE dilutions from TiterTubes to duplicate wells in MaxiSorp plate. Change pipet tips appropriately to avoid cross-contamination. Cover plate with plate sealing film. Incubate for 2 hours at ambient temperature.

9. At the end of the 2-hour incubation step, prepare Secondary Antibody solution
    - Briefly spin the **Secondary Antibody (0.474 mg/mL)** vial and gently mix by pipetting up and down
    - **Dilute 1:474 in Assay Diluent to target 1 µg/mL.**
    - **Example: For one full plate, add 25.4 µL of Secondary Antibody to 12 mL Assay Diluent**

10. Empty contents from the plate and wash 3 times (each time 300 µL/well) with Wash Buffer using automatic plate washer or multi-channel pipette.

11. Add 100 µL/well of Secondary Antibody solution to plate. Cover plate with plate sealing film. Incubate for 1 hour at ambient temperature.
12. At the end of the 1-hour incubation step, prepare Detection Reagent solution
    - Briefly spin the **Detection Reagent** vial and gently mix by pipetting up and down
    - **Dilute Detection Reagent 1:4,000**
    - **Example: For one full plate, add 3 µL of Detection Reagent to 12 mL Assay Diluent**

13. Empty contents from the plate and wash 3 times (each time 300 µL/well) with Wash Buffer using automatic plate washer or multi-channel pipette.

14. Add 100 µL/well of Detection Reagent solution to plate. Cover plate with plate sealing film. Incubate for 1 hour at ambient temperature, shielded from light.
15. During this time, equilibrate TMB substrate to ambient temperature, shielded from light.

16. Empty contents from the plate and wash 3 times (each time 300 µL/well) with Wash Buffer using automatic plate washer or multi-channel pipette.

17. Add 100 µL/well of TMB substrate. Incubate plate at ambient temperature, shielded from light. Start timer for 30 min color development.
18. Immediately following the 30 min color development, place plate in the plate reader programmed to shake the plate for 5 sec prior to end-point read at 650 nm wavelength.
19. Prepare a standard curve from the data produced from the serial dilutions with concentrations on the x axis (log scale) vs. absorbance on the y axis (linear). Interpolate the concentration of the Unknown Samples from the standard curve.

Notes regarding plate washing:

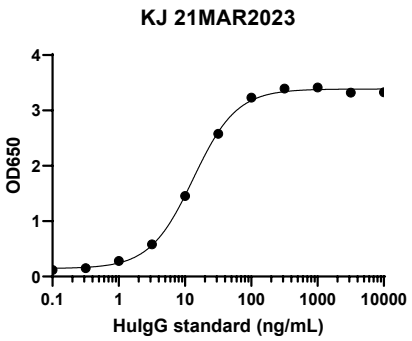
- ELISA Wash Buffer (1X DPBS + 0.05% TWEEN-20):
    - Dissolve one bottle of DPBS powder in deionized water to prepare 10 liters of 1X DPBS
    - Add 5 mL TWEEN-20 to 10 L of 1X DPBS
    - Gently mix
- Use BioTek plate washer model ELx405, "COSTAR_FLAT" program (Number of cycles: 3; Volume wash buffer: 300 µL/well).
- Empty the MaxiSorp plate's content into biohazard container and blot on paper towels
- Wash plate using "COSTAR_FLAT" program
- Tap plate on paper towels to remove any residual liquid.
- Immediately add solution to the wells. Do not let the wells dry for extended time.

7. Example Template and Standard Curve

EXAMPLE OF PLATE TEMPLATE

	1	2	3	4	5	6	7	8	9	10	11	12
A	STD 10,000	STD 3,162	STD 1,000	STD 316	STD 100	STD 31.6	STD 10.0	STD 3.2	STD 1.0	STD 0.32	STD 0.10	Blank
B												
C	Spl 1 Dil.1	Spl 1 Dil.2	Spl 1 Dil.3	Spl 4 Dil.1	Spl 4 Dil.2	Spl 4 Dil.3	Spl 7 Dil.1	Spl 7 Dil.2	Spl 7 Dil.3	Spl 10 Dil.1	Spl 10 Dil.2	Spl 10 Dil.3
D												
E	Spl 2 Dil.1	Spl 2 Dil.2	Spl 2 Dil.3	Spl 5 Dil.1	Spl 5 Dil.2	Spl 5 Dil.3	Spl 8 Dil.1	Spl 8 Dil.2	Spl 8 Dil.3	Spl 11 Dil.1	Spl 11 Dil.2	Spl 11 Dil.3
F												
G	Spl 3 Dil.1	Spl 3 Dil.2	Spl 3 Dil.3	Spl 6 Dil.1	Spl 6 Dil.2	Spl 6 Dil.3	Spl 9 Dil.1	Spl 9 Dil.2	Spl 9 Dil.3	Spl 12 Dil.1	Spl 12 Dil.2	Spl 12 Dil.3
H												

EXAMPLE OF STANDARD CURVE



A = 0.144
B = 1.328
C = 13.29
D = 3.387

DATA ANALYSIS

Softmax software is used to calculate Human IgG (ng/mL) detected in Unknown Samples, based on the 4PL standard curve, tested on every plate, using the following equation:

$$X = C * \left(\frac{A - Y}{Y - D}\right)^{(1/B)}$$

X = ng/mL
Y = Absorbance Value (OD 650 nm)

A = Lower asymptote
B = Slope
C = Inflection point
D = Upper asymptote

8. Recovery of Human IgG spiked in rodent serum

	HuIgG standard diluted in			
HuIgG	Assay	Naïve Mouse Serum		
ng/mL	Diluent	1:100	1:1,000	1:10,000
10000.00	3.329	3.336	3.326	3.315
3162.28	3.322	3.325	3.346	3.327
1000.00	3.416	3.359	3.372	3.250
316.23	3.394	3.325	3.346	3.212
100.00	3.233	3.134	3.103	3.043
31.62	2.579	2.474	2.457	2.266
10.00	1.456	1.335	1.337	1.301
3.16	0.582	0.585	0.572	0.543
1.00	0.281	0.308	0.268	0.274
0.32	0.153	0.159	0.161	0.159
0.10	0.120	0.129	0.131	0.135
	Mean OD650 of duplicate wells			

	HuIgG standard diluted in			
HuIgG	Assay	Naïve Mouse Serum		
ng/mL	Diluent	1:100	1:1,000	1:10,000
10000.00	100%	100%	100%	100%
3162.28	100%	100%	101%	100%
1000.00	100%	98%	99%	95%
316.23	100%	98%	99%	95%
100.00	100%	97%	96%	94%
31.62	100%	96%	95%	88%
10.00	100%	92%	92%	89%
3.16	100%	101%	98%	93%
1.00	100%	110%	95%	98%
0.32	100%	104%	105%	104%
0.10	100%	108%	109%	113%
	Comparing OD650 to the standard diluted in Assay Diluent			

	HuIgG standard diluted in			
HuIgG	Assay	Naïve Rat Serum		
ng/mL	Diluent	1:100	1:1,000	1:10,000
10000.00	3.182	3.177	3.220	3.146
3162.28	3.179	3.150	3.178	3.160
1000.00	3.255	3.175	3.211	3.116
316.23	3.197	3.157	3.228	3.067
100.00	3.000	2.963	2.938	2.870
31.62	2.468	2.399	2.354	2.243
10.00	1.431	1.424	1.332	1.319
3.16	0.623	0.641	0.616	0.599
1.00	0.279	0.321	0.257	0.251
0.32	0.161	0.190	0.170	0.155
0.10	0.122	0.163	0.139	0.139
	Mean OD650 of duplicate wells			

	HuIgG standard diluted in			
HuIgG	Assay	Naïve Rat Serum		
ng/mL	Diluent	1:100	1:1,000	1:10,000
10000.00	100%	100%	101%	99%
3162.28	100%	99%	100%	99%
1000.00	100%	98%	99%	96%
316.23	100%	99%	101%	96%
100.00	100%	99%	98%	96%
31.62	100%	97%	95%	91%
10.00	100%	100%	93%	92%
3.16	100%	103%	99%	96%
1.00	100%	115%	92%	90%
0.32	100%	118%	106%	96%
0.10	100%	134%	114%	114%
	Comparing OD650 to the standard diluted in Assay Diluent			