ab283987 – Human Immunoglobulin J Chain ELISA Kit

For the quantitative measurement of Immunoglobulin J (IGJ) in Human plasma, serum, and cell culture supernatant samples.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
www.abcam.com/ab283987

Storage and Stability: Store kit at +4°C immediately upon receipt, apart from the Standard, SP Conjugate, and Biotinylated Antibody which should be stored at -20°C. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Materials Supplied

Item	Quantity	Storage Condition
Human Immunoglobulin J Chain Microplate	96 wells	4°C
100X Streptavidin-Peroxidase (SP) Conjugate	80 µL	-20°C
10X Diluent M Concentrate	20 mL	4°C
1X Standard Diluent	2 mL	4°C
20X Wash Buffer Concentrate	2 x 30 mL	4°C
50X Biotinylated Human Immunoglobulin J Chain Antibody	1 vial	-20°C
Chromogen Substrate	7 mL	4°C
Immunoglobulin J Chain Standard	2 vial	-20°C
Sealing Tapes	3 units	4°C
Stop Solution	11 mL	4°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Microplate reader capable of measuring absorbance at 450 nm
- Pipettes (1-20 µl, 20-200 µl, 200-1000 µl, and multiple channel)
- Deionized or distilled reagent grade water

Reagents Preparation

Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells.
Prepare only as much reagent as is needed on the day of the experiment.
If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.
Δ Note: Concentration of the kit components are lot-specific, and the end user should always refer to the vial label.

10X Diluent M Concentrate: Dilute the Diluent M Concentrate 10-fold with reagent grade water to produce a 1X solution. Store for up to 30 days at 4°C.

50X Biotinylated Human Immunoglobulin J Chain Antibody: Spin down the antibody briefly and dilute the desired amount of the antibody 50-fold with Diluent M to produce a 1X solution. The undiluted antibody should be stored at -20°C.

20X Wash Buffer Concentrate: Dilute the Wash Buffer Concentrate 20-fold with reagent grade water to produce a 1x solution.

100X SP Conjugate: Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 100-fold with Diluent M to produce a 1X solution. The undiluted conjugate should be stored at -20°C.

Standard Preparation

Always prepare a fresh set of standards for every use.
Prepare serially diluted standards immediately prior to use.
Any remaining standard should be stored at -20°C after reconstitution and used within 30 days.
Reconstitution of the Immunoglobulin J Chain Standard vial to prepare a 40 ng/mL Stock Standard:

- First consult the Immunoglobulin J Chain Standard vial to determine the mass of protein in the vial.
- Calculate the appropriate volume of Standard Diluent to add when resuspending the Immunoglobulin J Chain Standard vial to produce a 40 ng/mL Immunoglobulin J Chain Stock Standard by using the following equation:
    - C_S = Starting mass of Immunoglobulin J Chain Standard (see vial label) (ng)
    - C_F = The 40 ng/mL Immunoglobulin J Chain Stock Standard final required concentration
    - V_D = Required volume of Standard Diluent for reconstitution (µL)
    - Calculate total required volume Standard Diluent for resuspension:

$$(C_S / C_F) \times 1{,}000 = V_D$$

Example: **Δ Note: This example is for demonstration purposes only. Please remember to check your standard vial for the actual amount of standard provided**.

CS = 20 ng of Immunoglobulin J Chain Standard in vial

CF = 40 ng/mL Immunoglobulin J Chain Stock Standard final concentration

VD = Required volume of Standard Diluent for reconstitution (80 ng / 100 ng/mL) x 1000 = 500 µL

- Reconstitute the Immunoglobulin J Chain Standard vial by adding the appropriate calculated amount V_D of Standard Diluent to the vial to generate the 40 ng/mL Immunoglobulin J Chain Stock Standard. Mix gently and thoroughly.
- Allow the reconstituted 40 ng/mL stock to sit for 10 minutes with gentle agitation prior to making subsequent dilutions

The following section describes the preparation of a standard curve.

- Label eight tubes 1 – 8.
- From the standard stock solution (40 ng/ml), dilute 5-fold with Diluent M to produce a 20 ng/ml standard working solution, in tube 1.
- To prepare Standard 2, dilute the working solution two-fold with Diluent M into tube 2 and mix gently.

- Using the table below as a guide, prepare subsequent serial dilutions. Diluent M serves as the zero standard (0 ng/mL).

Standard #	Immunoglobulin J Chain (ng/mL)
1	20
2	10
3	5.0
4	2.5
5	1.25
6	0.625
7	0.313
8	0.0

- Aliquot remaining stock solution to limit repeated freeze-thaw cycles. This solution should be stored at -20°C and used within 30 days.

Sample Preparation

Plasma: Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant (EDTA or Heparin can also be used as an anticoagulant). Centrifuge samples at 3000 x g for 10 minutes and collect plasma. Plasma samples are suggested for use at 1X; however, users should determine optimal dilution factors depending on application needs. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

Serum: Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3000 x g for 10 minutes and remove serum. Serum samples are suggested for use at 1X; however, users should determine optimal dilution factors depending on application needs. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles

Cell Culture Supernatant: Centrifuge cell culture media at 1500 rpm for 10 minutes at 4°C to remove debris and collect supernatant. If necessary, dilute samples into Diluent M; users should determine optimal dilution factors depending on application needs. The undiluted samples can be stored at -80°C. Avoid repeated freeze-thaw cycles

Plate Preparation

The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
Unused plate strips should be immediately returned to the foil pouch containing the desiccant pack, resealed and stored at 4°C.
For each assay performed, a minimum of two wells must be used as the zero control.
For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).

Assay Procedure

Equilibrate all materials and prepared reagents to room temperature prior to use.
We recommend that you assay all standards, controls and samples in duplicate

1. Add 50 µL of Human IGJ Standard or sample to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last addition.
2. Wash the microplate manually or automatically using a microplate washer. Invert the plate and decant the contents; tap 4-5 times on absorbent material to completely remove the liquid. If washing manually, wash five times with 200 µL of Wash Buffer per well. Invert the plate each time and decant the contents; tap 4-5 times on absorbent material to completely remove the liquid. If using a microplate washer, wash six times with 300 µL of Wash Buffer per well; invert the plate and tap 4-5 times on absorbent material to completely remove the liquid.
3. Add 50 µL of Biotinylated Human IGJ Antibody to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 2 hours.
4. Wash the microplate as described above.
5. Add 50 µL of SP Conjugate to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
6. Wash the microplate as described above.
7. Add 50 µL of Chromogen Substrate to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Incubate for 25 minutes or until the optimal blue color density develops.
8. Add 50 µL of Stop Solution to each well. The color will change from blue to yellow. Gently tap plate to ensure thorough mixing. Break any bubbles that may have formed.
9. Read the absorbance on a microplate reader at a wavelength of 450 nm immediately. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only. Please note that some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

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Additional information

CALCULATION

1. Calculate the mean value of the duplicate or triplicate readings for each standard and sample.
2. To generate a standard curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm 6 absorbance (OD) on the y-axis. The best fit line can be determined by regression analysis using log-log or four-parameter logistic curve fit.
3. Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

TYPICAL DATA

Typical data provided for demonstration purposes only.

Standard #	ng/mL	OD	Average OD
1	20	1.900 1.960	1.930
2	10	1.690 1.798	1.744
3	5.0	1.408 1.334	1.371
4	2.5	0.989 1.025	1.007
5	1.25	0.690 0.648	0.669
6	0.625	0.450 0.426	0.438
7	0.313	0.291 0.301	0.296
8	0.0	0.130 0.124	0.127

PERFORMANCE CHARACTERISTICS

1. This assay recognizes both natural and recombinant human IGJ.
2. The minimum detectable dose of human IGJ as calculated by 2SD from the mean of a zero standard was established to be 0.13 ng/mL.
3. Intra-assay precision was determined by testing three plasma samples twenty times in one assay.
4. Inter-assay precision was determined by testing three plasma samples in twenty assays.

	Intra-Assay Precision	Inter-Assay Precision
CV (%)	4.7	10.9

RECOVERY

Standard Added Value: 0.625 – 5 ng/mL
Recovery: 95-102%
Average Recovery: 99%

LINEARITY

Average Percentage of Expected Value (%)		
Sample Dilution	**Plasma**	**Serum**
1x	90%	91%
2x	105%	101%
4x	96%	109%

CROSS REACTIVITY

Species	Cross-Reactivity (%)
Canine	50%
Bovine	None
Equine	10%
Monkey	90%
Mouse	40%
Rat	50%
Swine	70%
Rabbit	None

No significant cross-reactivity observed with human IgA, IgA1, IgA2, IgD, IgE, IgG, IgG1, IgG2, IgG3, IgG4, IgLL1, and IgM proteins.
10% FBS in culture media will not affect the assay.

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)

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