ProQuantum™ Human IgA Immunoassay Kit

Catalog Number A45729 (96 reactions), A457295 (5 × 96 reactions)

Pub. No. MAN0018839 Rev. D.0

Product description

The ProQuantum™ Human IgA Immunoassay Kit is designed to quantify human IgA protein in serum, plasma, and cell culture supernatant using a qPCR instrument. The assay is expected to work with other sample types, but may need protocol modifications for optimal results. This protocol describes how to run an assay using an entire 96-well plate with 5-µL sample volumes. To customize plate design, or for protocols using samples at half volume (2 µL), go to apps.thermofisher.com/apps/proquantum.

Contents and storage

Upon receipt, store the kit at –20°C. Expires one year from date of receipt when stored as instructed.

Contents	Cat. No. A45729 (96 rxn)
Human IgA Antibody-conjugate A	15 µL
Human IgA Antibody-conjugate B	15 µL
Human IgA Protein Standard, lyophilized	2 vials
Master Mix	5 mL
Ligase	40 µL
Antibody-conjugate Dilution Buffer	700 µL
Assay Dilution Buffer	7.5 mL

General guidelines

- Review the *ProQuantum™ Technical Guide* at thermofisher.com for details before starting the procedure.
- Wear gloves, use DNase/RNase/pyrogen free plastic ware, and practice proper RNA handling techniques.
- Centrifuge vials before pipetting to ensure the contents are at the bottom of the tube.
- Thaw all reagents **except Ligase** at room temperature. Keep Ligase and thawed reagents on ice.
- Do not vortex plates.
- Use a plate seal hand tool to ensure complete adherence to avoid any evaporation or contamination.
- Use best practices with pipetting to minimize CV.
- If any particulate matter is present in the sample, centrifuge or filter sample before performing the assay.

Required materials not provided

Product	Cat. No. [1]
Calibrated pipettes and low retention filter tips	MLS
96-well plate cold block	Fisher 50-589-601
96-well assay plate and 96-well working plate	MLS
25-mL reagent reservoir × 2	Fisher 14387071
Microtiter plate adhesive seals × 2	Fisher 4306311
Microtiter plate adhesive seal, optical grade	Fisher AB1170
Plate seal hand tool	4333183
Centrifuge with swinging bucket rotor for 96-well plates, and microcentrifuge	MLS
96-well plate rack	Fisher 05-541-80
RNase-free 1.5-mL microcentrifuge tube	AM12400
Sterile 15-mL conical tube	339651

[1] MLS: major laboratory supplier.

Prepare reagents

Reconstitute standard

1. Reconstitute one vial of protein standard with Assay Dilution Buffer to 2,000 ng/mL. See label on the vial for reconstitution volume. Mix by inverting the vial five times. **Do not vortex**.
2. Incubate for 15 minutes at room temperature.

Prepare working plate (on cold block 4°C)

Mix antibody-conjugates

1. Add the following components to a 1.5-mL microcentrifuge tube, then mix by pipetting up and down.

Component	Volume
Antibody-conjugate A	12 µL
Antibody-conjugate B	12 µL
Antibody-conjugate Dilution Buffer	696 µL

2. Dispense ≥80 µL of antibody-conjugate mixture to each well in one column of the working plate.

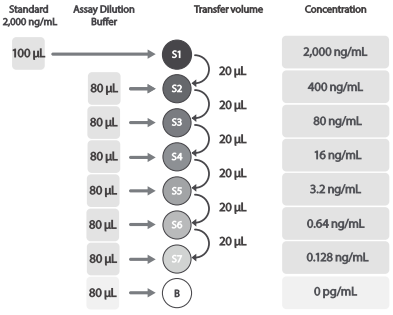
Dilute samples

1. Dilute samples containing a high abundance of target protein 1:10,000 with PBS (not supplied with kit).
   a. Dilute samples 1:100 with PBS, pH 7.4.
   b. Dilute the diluted samples 1:100 with PBS, pH 7.4.

   This dilution factor is a recommended starting point. Adjust the value to determine the optimal conditions for your samples.
2. Add 5 µL of sample to 45 µL of Assay Dilution Buffer in the working plate, then mix by pipetting up and down.

Create standard curve

1. Add 80 µL of Assay Dilution Buffer to wells S2–S7 in one column of the working plate reserved for standards.
2. Mix reconstituted standard by gently inverting the vial five times. Transfer 100 µL of protein standard to the first well (S1).
3. Make serial dilutions of the standard by transferring 20 µL from well S1 to well S2. Continue serial dilution to well S7. The Background well (B) should only contain Assay Dilution Buffer. Mix wells thoroughly. Change pipette tips between dilution steps.



Note: The standard curve can be modified to extend the range. See "Example of extended standard curve" on page 2.

4. Seal the working plate with an adhesive seal using the plate seal hand tool.

5. Mix by striking the side of the plate against the palm of your hand three times. Rotate the plate and strike the other side of the plate against your palm three times.
6. Centrifuge at 3,000 × *g* for 1 minute to collect the liquid at the bottom of all wells.
7. Discard remaining unused reconstituted standard.

Run assay

Bind analyte (1 hour)

Keep the assay plate on a plate rack except during mixing or centrifugation steps.

1. Transfer 5 µL of antibody-conjugate mixture from the working plate by multichannel pipette to all assay wells in the assay plate.
2. Transfer 5 µL of standards or diluted samples from the working plate to the appropriate wells in the assay plate. Mix thoroughly by pipetting up and down several times.
3. Seal the assay plate with an adhesive seal. Mix by striking the plate. Centrifuge at 3000 × *g* for 1 minute.
4. Incubate the assay plate for 1 hour at room temperature.

   (*Optional*) Incubate the assay plate overnight at 4°C.

Perform qPCR

1. Add 5 mL of Master Mix and 30 µL of Ligase to a 15-mL conical tube. Invert the tube five times, then pour the qPCR reaction mixture into a reagent reservoir.
2. Add 40 µL of qPCR reaction mixture to all assay wells. Mix by pipetting up and down. Avoid introducing bubbles in the wells.
3. Seal the assay plate with an optical plate seal. Ensure there is complete adhesion using the plate seal hand tool.
4. Mix by striking plate, then centrifuge at 3,000 × *g* for 1 minute.
5. Create a new Experiment on the qPCR instrument.

Parameter	Settings for Applied Biosystems™ instruments
Experiment type	Standard Curve or Quantitation - Standard Curve
Reagents	TaqMan™ reagents
Reporter dye	FAM
Quencher	NFQ-MGB [1]
Passive reference	ROX
Assign wells	Define all wells of the 96-well plate as Unknown
Threshold	0.2
Baseline	3–15

[1] For instruments without this option, enter "None" or "Non-fluorescent".

6. Input the PCR Method based on your instrument block type.

Step	Temp (°C)[1]	Time (by block type)		Stage
		Standard	Fast[2]	
Ligation	25	20 min	20 min	Hold
Ligase inactivation	95	2 min	2 min	Hold
Denaturation	95	15 s	1 s	40 cycles
Annealing/extension	60	1 min	20 s	

[1] Set ramp rate to 2°C/sec

[2] Use default values for 7500, 7500 Fast, 7900HT, or non-Applied Biosystems instruments (e.g., 3 s denaturation and 30 s annealing/extension).

7. Save the Experiment as a template. Run the template.

   Note: Reuse the saved template for subsequent assays.

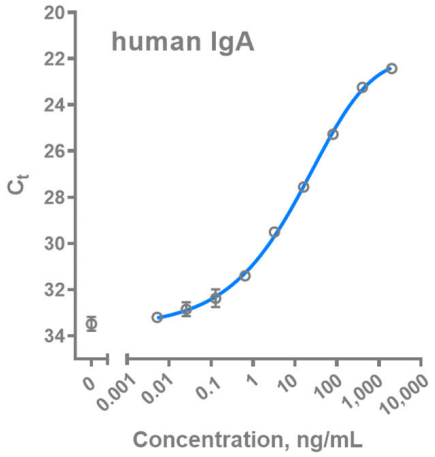
Import run data

1. Save the run data in .eds or .sds format (or .csv format for non-Applied Biosystems instruments) to a USB memory device, or directly to the Connect cloud-based platform.

   Note: If your qPCR instrument is connected to a computer, download the run data from the computer and **not** directly from the qPCR instrument.
2. Import .eds, .sds., or .csv files to the ProQuantum™ software (available at apps.thermofisher.com/apps/proquantum) to generate standard curves and determine sample concentrations.

Example of extended standard curve

The data in this section is provided as an example of a typical standard curve. Prepare your own standard curve for each assay plate.



Concentration (ng/mL)	Average C_t
2,000	22.4
400	23.2
80	25.3
16	27.5
3.2	29.5
0.64	31.4
0.128	32.4
0.0256	32.8
0.00512	33.2
0	33.5

Limited product warranty

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The information in this guide is subject to change without notice.