SARTORIUS

Product Guide

iQue® SARS-CoV-2 (IgG, IgM and IgA) Kit

Product Information

Presentation, Storage and Stability

The iQue® SARS-CoV-2 (IgG, IgM and IgA) Kit contains ready-to-use reagents for simultaneous detection of human IgG, IgM and IgA antibodies specific for the receptor binding domain (RBD) of SARS-CoV-2 Spike protein in a single well using the iQue® VBR platform. The kit is available in 1x96, 5x96, 1x384, 5x384-well formats, and includes SARS-CoV-2 Spike RBD-coupled Beads, SARS-CoV-2 Antibody Detection Cocktail, Anti-SARS-CoV-2 Spike RBD Standards (human IgG, IgM and IgA), Assay Diluent, Wash Buffer, and USB flash drive containing analysis templates. Upon receipt, the SARS-CoV-2 Spike RBD-coupled Beads and Anti-SARS-CoV-2 Spike RBD Standards (human IgG, IgM and IgA) should be stored at -20°C, and the other kit components should be stored at 2-8°C. When stored at the above temperatures, the kit is stable for at least 6 months upon receipt. Refer to the reagent labels for the exact expiration date.

Product Name	Cat. No.	Format
iQue® SARS-CoV-2 (IgG, IgM and IgA) Kit	BA-97125	1 x 96-well
	BA-97126	5 x 96-well
	BA-97127	1x384-well
	BA-97128	5x384-well

Table 1. Product Information

Kit Components	Cat. No. BA-97125 1x96-well	Cat. No. BA-97126 5 x 96-well	Cat. No. BA-97127 1x384-well	Cat. No. BA-97128 5x384-well	Storage	Stability
SARS-CoV-2 Spike RBD-coupled Beads	1 vial (50 µL)	5 vials (50 µL)	1 vial (100 µL)	5 vials (100 µL)	-20°C	Refer to the reagent labels for expiration date.
SARS-CoV-2 Antibody Detection Cocktail	1 bottle (2 mL)	5 bottles (2 mL)	1 bottle (5.4 mL)	5 bottles (5.4 mL)	2–8°C	
Anti-SARS-CoV-2 Spike RBD Standard (human IgG)[a]	1 vial (50 µL)	1 vial (250 µL)	1 vial (50 µL)	1 vial (250 µL)	-20°C	
Anti-SARS-CoV-2 Spike RBD Standard (human IgM)[b]	1 vial (50 µL)	1 vial (250 µL)	1 vial (50 µL)	1 vial (250 µL)	-20°C	
Anti-SARS-CoV-2 Spike RBD Standard (human IgA)[c]	1 vial (50 µL)	1 vial (250 µL)	1 vial (50 µL)	1 vial (250 µL)	-20°C	
Assay Diluent	1 bottle (25 mL)	1 bottle (125 mL)	1 bottle (50 mL)	1 bottle (250 mL)	2–8°C	
Wash Buffer	1 bottle (25 mL)	1 bottle (125 mL)	1 bottle (50 mL)	1 bottle (250 mL)	2–8°C	

Table 2. Kit Components and Storage

Note: Safety data sheet (SDS) can be found on our website **www.sartorius.com**. A kit product guide and a USB key with assay templates are also included in the kit package.

[a] Anti-SARS-CoV-2 Spike RBD Neutralizing Antibody, Recombinant Human IgG1 Monoclonal Antibody
[b] Anti-SARS-CoV-2 Spike RBD Neutralizing Antibody, Recombinant Human IgM Monoclonal Antibody (without J chain)
[c] Anti-SARS-CoV-2 Spike RBD Neutralizing Antibody, Recombinant Human IgA1 Monoclonal Antibody (without J chain)

Parameter	IgG	IgM	IgA
iQue® Detection Channel	RL-1	BL-3	BL-1
Lower Limit of Detection (pg/mL)	31	61	61
Intra-assay precision, %CV		<10%	
Inter-assay precision, %CV		<15%	

Table 3. Assay Performance Characteristics
Note: CV: coefficient of variation

Background

COVID-19 is a respiratory infectious disease caused by the single-stranded RNA coronavirus, SARS-CoV-2, and continues to be a global public health threat. The SARS-CoV-2 Spike RBD is responsible for viral entry into host cells via binding to the angiotensin-converting enzyme 2 (ACE2), and is the primary target of the COVID-19 vaccines currently in use. Antibodies against the SARS-CoV-2 Spike RBD have been shown to have potent antiviral activity and to correlate with protective immunity against the virus. Serological testing to assess levels of the three major isotypes (IgG, IgM and IgA) of antibodies specific for SARS-CoV-2 Spike RBD is valuable in characterizing the immune response against infection in COVID-19 patients, determining the vaccination status of an individual, and detecting previous infection in asymptomatic individuals.

The iQue® SARS-CoV-2 (IgG, IgM and IgA) kit is a robust bead-based immunoassay designed for detection of anti-SARS-CoV-2 Spike RBD antibodies (IgG, IgM and IgA) that are produced by the immune system in response to the viral infection or vaccination. With unique surface coating, the SARS-CoV-2 Spike RBD-coupled Beads effectively capture anti-SARS-CoV-2 Spike RBD antibodies from human blood samples (serum and plasma). The bead-bound antibodies are subsequently detected by a secondary antibody cocktail containing anti-human IgG, IgM and IgA antibodies which are labeled with different fluorophores, enabling multiplexed analysis of all three isotypes (IgG, IgM and IgA) of anti-SARS-CoV-2 Spike RBD antibodies in a single sample with a streamlined workflow. The assay is optimized to run on the iQue® platform with VBR configuration combining high throughput sampling and analysis for SARS-CoV-2 serological surveillance using flow cytometry detection capabilities. Pre-set templates for gating strategy and analysis are included in the kit for ease of use.

Recommended Use

The iQue® SARS-CoV-2 (IgG, IgM and IgA) Kit is used for qualitative and quantitative detection of IgG, IgM and IgA antibodies against SARS-CoV-2 Spike RBD in human blood samples (serum and plasma). The kit has been validated using samples from both COVID-19 diagnosed patients and vaccinated personnel. This product is for research use only.

- It is recommended to pre-dilute the test samples 100–500 fold using the included Assay Diluent for the assay.
- Aliquot the individual Anti-SARS-CoV-2 RBD Standards (human IgG, IgM and IgA) prior to the first assay if needed, and store at -20°C. Avoid freeze/thaw cycles.
- For quantitative accuracy, it is recommended to run antibody standards with each assay.
- For optimal quantification of anti-IgA antibody levels, plates should be acquired on the iQue® platform within two hours of assay completion when stored at 4°C.

COVID Kit Workflow

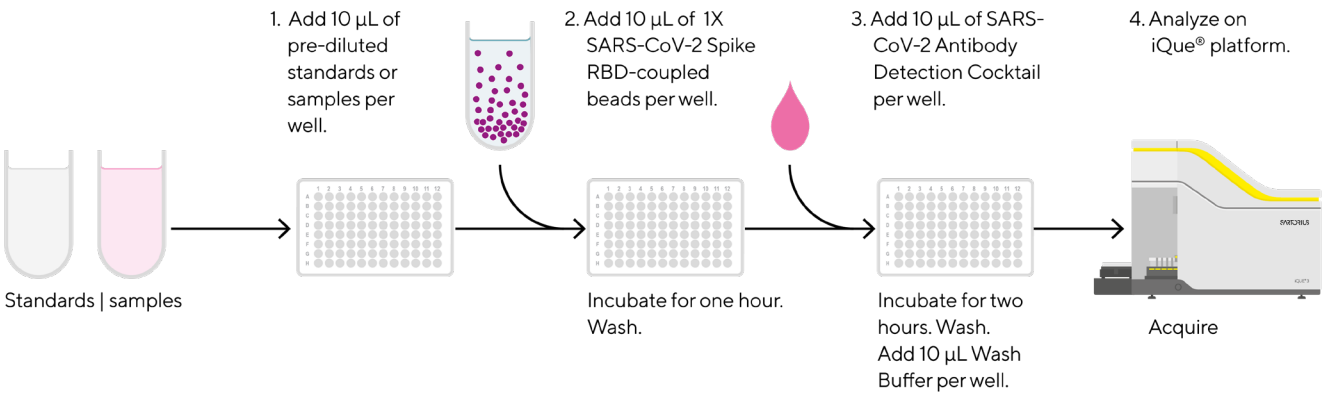

Figure 1: iQue® SARS-CoV-2 (IgG, IgM and IgA) Kit workflow

Protocol and Procedure

1.0 Prepare Reagents and Samples

1.1 Allow all the kit components and test samples to come to room temperature (RT) prior to assay. Briefly centrifuge all vials before use to prevent reagent loss.

1.2 Prepare 1:3 serial dilutions of the three anti-SARS-CoV-2 Spike RBD Standards (human IgG, IgM and IgA) as shown in **Figure 2**. For easy transfer into assay plates, dilutions are arranged from low to high concentrations as follows:

1.2.1 For a 96-well plate assay, prepare 12 microcentrifuge tubes and label them #1 –#12. For a 384-well plate assay, prepare 16 microcentrifuge tubes and label them #1–#16. You may also use an empty 96-well plate or 12-channel pipette reservoir instead of microtubes.

1.2.2 Add 40 µL of Assay Diluent to tubes #1–#11 (for 96-well plate assay) or tubes #1–#15 (for 384-well plate assay).

1.2.3 Combine 20 µL of each anti-SARS-CoV-2 Spike RBD Standards (human IgG, IgM and IgA) into tube #12 (for 96-well plate assay) or #16 (for 384-well plate assay), and mix by pipetting. This is the highest antibody concentration (1,000,000 pg/mL for each isotype) for the standard curve.

1.2.4 Transfer 20 µL of standard from tube #12 to tube #11 (for 96-well plate assay) or from tube # 16 into tube #15 (for 384-well plate assay). Pipette gently for thorough mixing.

1.2.5 Continue serial dilution transfer and mixing until tube #2 for either a 96-well or 384-well plate assay.

1.2.6 Do not transfer any standard to tube #1 for either a 96-well or 384-well plate assay. This tube will serve as the negative control without antibody standard.

1.3 Dilute serum or plasma samples in Assay Diluent at least 100-fold. Mix thoroughly.

1.4 Dilute SARS-CoV-2 Spike RBD-coupled Beads.

1.4.1 Vigorously vortex beads for at least 30 seconds.

1.4.2 Dilute beads 50-fold in Assay Diluent (For example, add 50 µL beads to 2.45 mL Assay Diluent). Mix thoroughly.

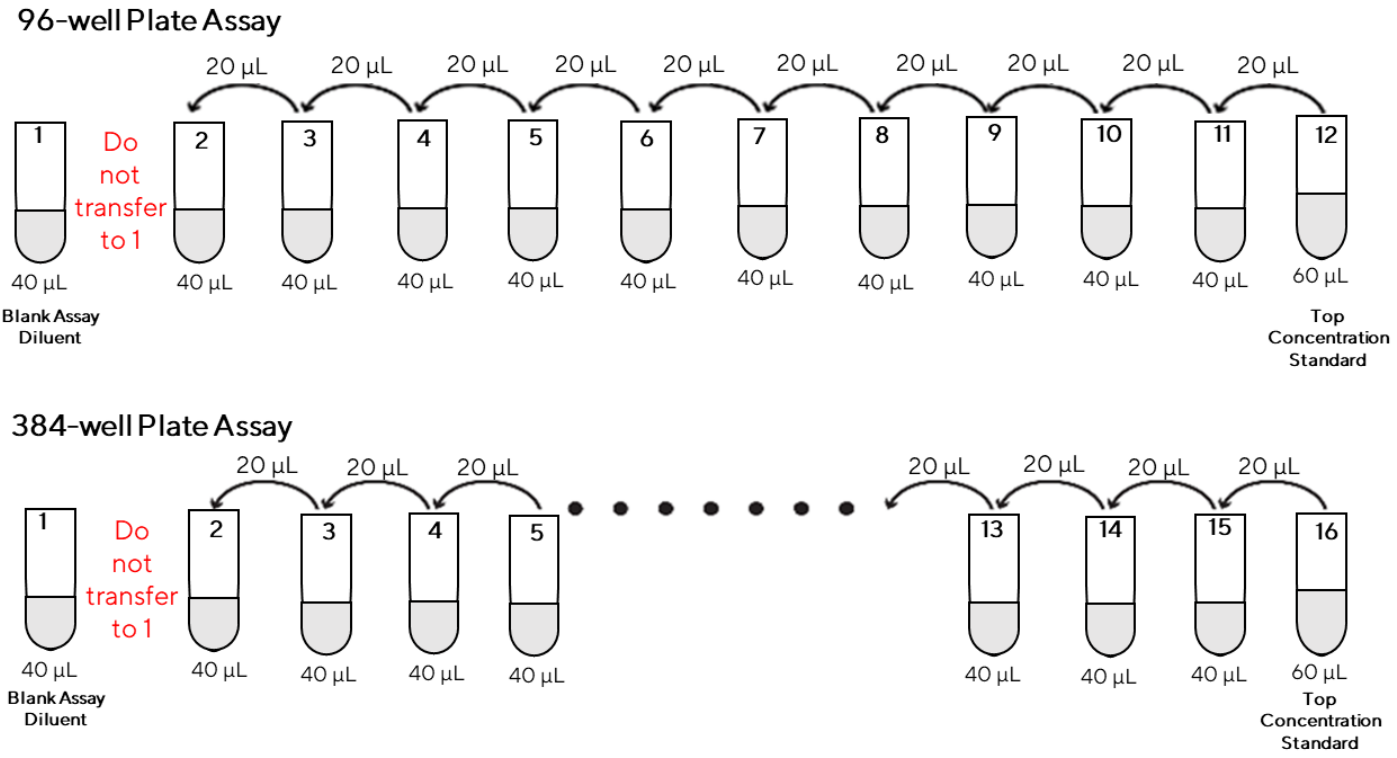

Figure 2: Preparation of Antibody Standards. Dilute combined antibody standards (IgG, IgM and IgA) 1:3 in Assay Diluent. Use 12 samples (96-well) or 16 samples (384). Note that tube #1 for both formats contains Assay Diluent alone.

2.0 Incubate SARS-CoV-2 Spike RBD-Coupled Beads with Antibody Standards and Samples

2.1 Transfer 10 µL of the combined anti-SARS-CoV-2 Spike RBD Standards (human IgG, IgM and IgA) or pre-diluted serum/plasma samples to each well of a 96-well or 384-well assay plate according to the plate map designation.

Note: The Standard set is preconfigured with the lowest value set to zero in the template provided. It is recommended to load standards in duplicate from low to high concentration in the direction of the plate read (96-well format: left to right; 384-well format: top to bottom). For iQue Forecyt® version 7.1 and later this is the default setting, however, for earlier versions, this format requires the "Reverse Series" box to be checked).

2.2 Vigorously vortex the pre-diluted SARS-CoV-2 Spike RBD-coupled Beads for at least 15 seconds (**Step 1.4**).

2.3 Add 10 µL of the beads to each assay well. Agitate the beads occasionally to prevent the beads from settling.

2.4 Give the assay plate a quick spin (300 x g, 5 seconds) and brief shake (2000 RPM, 20 seconds) using the iQue® plate shaker to ensure that all samples are thoroughly mixed at the well bottom.

2.5 Cover the plate and incubate at RT for one hour.

2.6 Add 100 µL Wash Buffer to each well of the 96-well plate or 50 µL Wash Buffer to each well of the 384-well plate.

2.7 Centrifuge at 1,100 x g for 5 minutes. Aspirate supernatant.

2.8 Resuspend beads in residual liquid with a strong shake (3000 RPM, 60 seconds) using the iQue® plate shaker.

3.0 Add SARS-CoV-2 Antibody Detection Cocktail

3.1 Add 10 µL of the SARS-CoV-2 Antibody Detection Cocktail to each assay well.

3.2 Give the assay plate a quick spin (300 x g, 5 seconds) and brief shake (2000 RPM, 20 seconds).

3.3 Cover the plate and incubate at RT for two hours; protect from light.

3.4 Add 100 µL Wash Buffer to each well of the 96-well plate or 50 µL Wash Buffer to each well of the 384-well plate.

3.5 Centrifuge at 1100 x g for 5 minutes. Aspirate supernatant.

3.6 Resuspend beads in residual liquid with a strong shake (3000 rpm, 60 seconds).

3.7 Add 10 µL of Wash Buffer to each assay well.

3.8 Give the assay plate a quick spin (300 x g, 5 seconds). The plate is now ready to run on the iQue®.

Note: The assay plate can be acquired immediately or sealed with a plate-sealer and stored at 4°C for later assay. Quantification of IgG and IgM antibodies are stable for up to 24 hours, and quantification of IgA antibody is stable for up to two hours.

4.0 Plate Acquisition and Data Analysis

4.1 Launch iQue Forecyt® Software.

4.2 Import the provided experiment template (included on USB key in the kit package).

4.3 Create a New Experiment using the provided template.

4.4 In the Design section, assign wells to Sample or desired well type.

4.5 In the Protocol section, adjust Sample Order on plate layout, if required (e.g., changing well acquisition to vertical instead of horizonal plate rows), adjust sip time as needed to ensure appropriate amounts of beads are acquired.

4.6 Click "Run" on the Controller window to acquire the plate.

4.7 Use the included template for gating strategy and subsequent analysis.

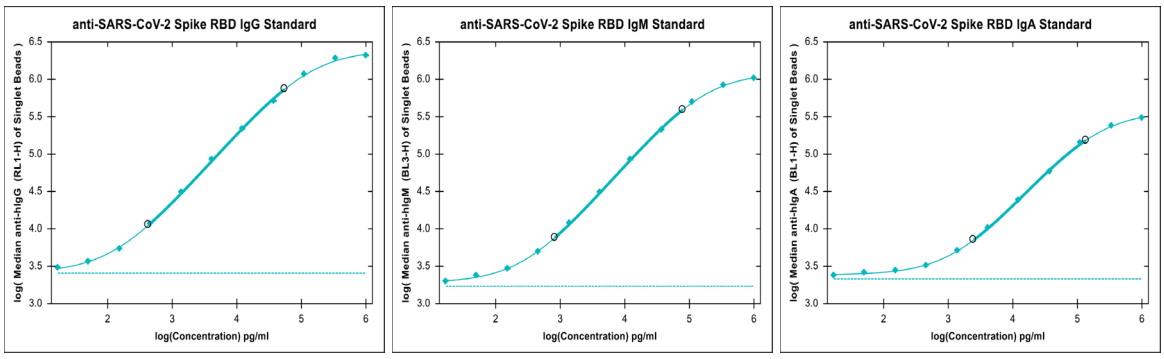

Figure 3: Standard curves of Anti-SARS-CoV-2 Spike RBD Standards included in kit. Anti-SARS-CoV-2 Spike RBD Standards (human IgG, IgM and IgA) were combined in a single tube at a starting concentration of 1 µg/mL, and then serially diluted 1:3 to generate standard curves for each antibody isotype.

Quick Guide

1.0 Reagent Preparation

- [ ] Allow included kit reagents and test samples to reach room temperature (RT) prior to assay.
- [ ] Prepare 1:3 serial dilution of Anti-SARS-CoV-2 Spike RBD Standards (human IgG, IgM, and IgA) and dilute test samples 100–500 fold.
- [ ] Dilute SARS-CoV-2 Spike RBD-coupled Beads 50-fold.

Prepare time _____ *Use Time* _____

2.0 Assay Protocol

- [ ] Add **10 µL** standards/samples and **10 µL** beads to each well of the assay plate. Quick Spin | Brief Shake*. **Incubate RT 1 Hour**

Start time _____ *Stop Time* _____

- [ ] Add **100 µL** (96-well) or **50 µL** (384-well) Wash Buffer to each well of the plate. Spin 1100 x g, 5 minutes. Aspirate Supernatant | Strong Shake.**
- [ ] Add **10 µL** SARS-CoV-2 Antibody Detection Cocktail to each well of the plate. Quick Spin | Brief Shake*. **Incubate RT 2 Hours, Dark**

Start time _____ *Stop Time* _____

- [ ] Add **100 µL/well** (96-well) or **50 µL** (384-well) Wash Buffer to each well of the plate. Spin 1100 x g, 5 minutes. Aspirate Supernatant | Strong Shake.**
- [ ] Add **10 µL** Wash Buffer to each well. Quick Spin. Acquire Data.

*Quick Spin: 300 x g, 5 sec. | Brief Shake: 2000 RPM, 20 sec. **Strong Shake: 3000 RPM, 60 sec.

Notes:

Sales and Service Contacts

For further information, visit www.sartorius.com

Sartorius BioAnalytical Instruments, Inc.

www.sartorius.com/ique

North America
Essen BioScience Inc.
300 West Morgan Road
Ann Arbor, Michigan, 48108
Telephone +1 734 769 1600
E-Mail: AskAScientist@sartorius.com
Online Store: shop.intellicyt.com

Europe
Essen BioScience Ltd.
Units 2 & 3 The Quadrant
Newark Close
Royston Hertfordshire
SG8 5HL
United Kingdom
Telephone +44 (0) 1763 227400
E-Mail: euorders.UK03@sartorius.com

APAC
Essen BioScience K.K.
4th floor Daiwa Shinagawa North Bldg.
1-8-11 Kita-Shinagawa
Shinagawa-ku, Tokyo
140-0001
Japan
Telephone: +81 3 6478 5202
E-Mail: orders.US07@sartorius.com

Publication No. #17206 Rev A.