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Product Guide

iQue® Human IgG Titer & Viability Kit

Product Information

Notice to Purchaser

The iQue® Human IgG Titer & Viability Kit is a member of the iQue® product line that has been extensively tested for live cell analysis applications. These screening kits are validated as complete screening assays and are optimized for use in high content screening applications. iQue® building blocks and reagents are designed for flexibility in multiplexing and incorporation into screening assays. iQue® reagent kits are specifically formatted for optimal performance on iQue® platforms.

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Limited Warranty

These products are offered under a limited warranty. The products are guaranteed to meet appropriate specifications described in the product insert at the time of shipment. Sartorius BioAnalytic Instruments, Inc., a Sartorius company, will provide product replacement for valid claims. All claims should be made within five (5) days of receipt of order.

Trademarks and Patents

List of Catalog Numbers

Product Name	Format	Cat. No.
iQue® Human IgG Titer & Viability Kit	1 x 384 wells	91142
iQue® Human IgG Titer & Viability Kit	5 x 384 wells	91143
iQue® Human IgG Titer & Viability Kit	20 x 384 wells	91144
iQue® Human IgG Titer & Viability Kit	50 x 384 wells	91145

Table 1: List of Catalog Numbers

Kit Contents

Product Name	Format
IgG Capture Beads	1 vial
Human FITC-IgG	1 vial
Control Human IgG	1 vial
iQue® Cell Membrane Integrity (R/Red) Dye	1 vial
Sample Reaction Buffer	1 bottle
BSA (lyophilized)	1 bottle

Table 2: Kit Contents

Note: Add Sample Reaction Buffer to BSA before use.

Detection Channels

Detector (nm)	Spectrum	Blue Laser (488 nm)		Red Laser (640 nm)	
533/30		(B/Green)	Bead Detection Channel		
585/40		(B/Yellow)			
670 nm LP		(B/Red)			
675/25 nm				(R/Red)	Cell Viability Detection Channel

Table 3. iQue® Standard Detector Channels

Detector (nm)	Spectrum	Violet Laser (405 nm)	Blue Laser (488 nm)		Red Laser (640 nm)	
445/45		(V/Blue)				
530/30		(V/Green)	(B/ Green)	Bead Detection Channel		
572/28		(V/ Yellow)	(B/ Yellow)			
615/24		(V/ Orange)	(B/ Orange)			
675/30		V/Red)	(B/Red)		(R/Red)	Cell Viability Detection Channel
780/60		(V/ Crimson)	(B/ Crimson)		(R/ Crimson)	

Table 4. iQue® 3 and iQue® Screener Plus Detector Channels

Note: For iQue® 3 and iQue® Screener Plus with VYB lasers, the bead detection channel is B/Green and the cell viability detection channel is V/Yellow. For iQue® 3 and iQue® Screener Plus with BR lasers, the bead detection channel and the cell viability detection channel are the same as iQue® 3 and iQue® Screener Plus with VBR lasers.

Note: Black boxes denote channels that are incompatible for use with the kit due to high spectral overlap that can not be compensated.

Materials Needed but Not Provided

- iQue® platform
- iQue Forecyt® Software
- iQue® QSol in the buffer station for tubing priming (Cat. No. 90283 or 90287)
- Centrifuge capable of spinning microcentrifuge tubes and | or 15 mL conical tubes at up to 8,000 x g
- Centrifuge capable of spinning microplates
- Vortex mixer
- Complete cell culture media
- Microcentrifuge tubes and | or 15 mL conical tubes
- 50 mL reagent reservoir (Example source: VWR®, Cat. No. 89094-680)
- Universal black lid (Example source: Corning®, Cat. No.3935) or foil to protect from light | evaporation
- 12-channel pipette reservoir (Example source: VWR®, Cat. No. 80092-466)
- Appropriate liquid handler or multi-channel pipette (**Appendix D**)

Table of Contents

Background

A key component of therapeutic monoclonal antibody development is identifying a cell line that secretes high levels of antibody into the culture supernatant. Early identification of high producing cell lines significantly increases the probability of success in downstream cell line manufacturing.

The iQue® Human IgG Titer & Viability Kit is a multiplexed assay for the iQue® platforms that simultaneously reports on IgG quantity, cell number and cell viability in each well for cell line production screening plates. These metrics enable the precise calculation of the IgG quantity per cell and per viable cell to better inform on cell productivity.

The iQue® Human IgG Titer & Viability Kit was designed for ease of use in multiplexing, enabling a straightforward workflow without sacrificing assay performance. Compared to other IgG quantitation methods, the iQue® solution offers several unique advantages:

- Simultaneous quantification of secreted IgG and viable cell count – Enables the precise quantitation of IgG per cell, IgG per viable cell, and cell viability and growth.
- Wide dynamic range (0.6 µg/mL to 20 mg/mL) – Enables transfer of cell culture samples directly into assay plates, without the need for dilution steps.
- No wash assay – Mix and read format minimizes screen time, cost, and variability.

Assay Principles

The iQue® Human IgG Titer & Viability Kit is a no-wash assay that enables the simultaneous quantification of secreted IgG per viable cell from each well of screening plates. This is a competition assay in which fluorescently labeled human IgG (human FITC-IgG) is added to samples containing secreted IgG and CHO production cells. The human FITC-IgG and non-labeled sample IgG compete for binding to IgG capture beads. The amount of IgG present in the sample is inversely proportional to the bead-associated FITC fluorescence. Cell viability is measured simultaneously in each well using cell membrane integrity dyes — fluorescent molecules that are cell impermeant. Healthy cells with intact cell membranes exclude the dye and are not fluorescent. Unhealthy cells with compromised membranes will allow entry of the dye into the intracellular space, where it then localizes to the nucleus and binds to DNA by intercalation.

Quantitative readouts from this assay can be measured as fluorescence intensity, or extrapolated to a concentration in solution via the use of a standard curve.

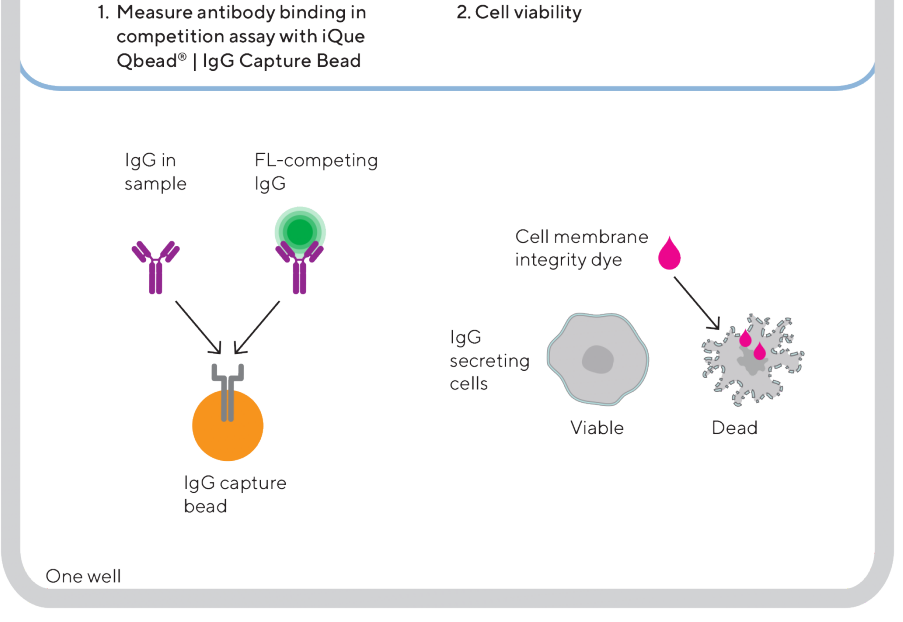


Figure 1: Figure 1. Assay principle of the iQue® Human IgG Titer & Viability Kit. The no-wash competition assay functions on the differential binding of cell-secreted IgG vs recombinant Human FITC-IgG to IgG Capture Beads. Samples with high IgG concentrations will exhibit a low fluorescence signal, whereas the absence or low concentration of solution IgG would result in a large fluorescence signal. The assay dynamic range is between 0.6 µg/mL to 20 mg/mL.

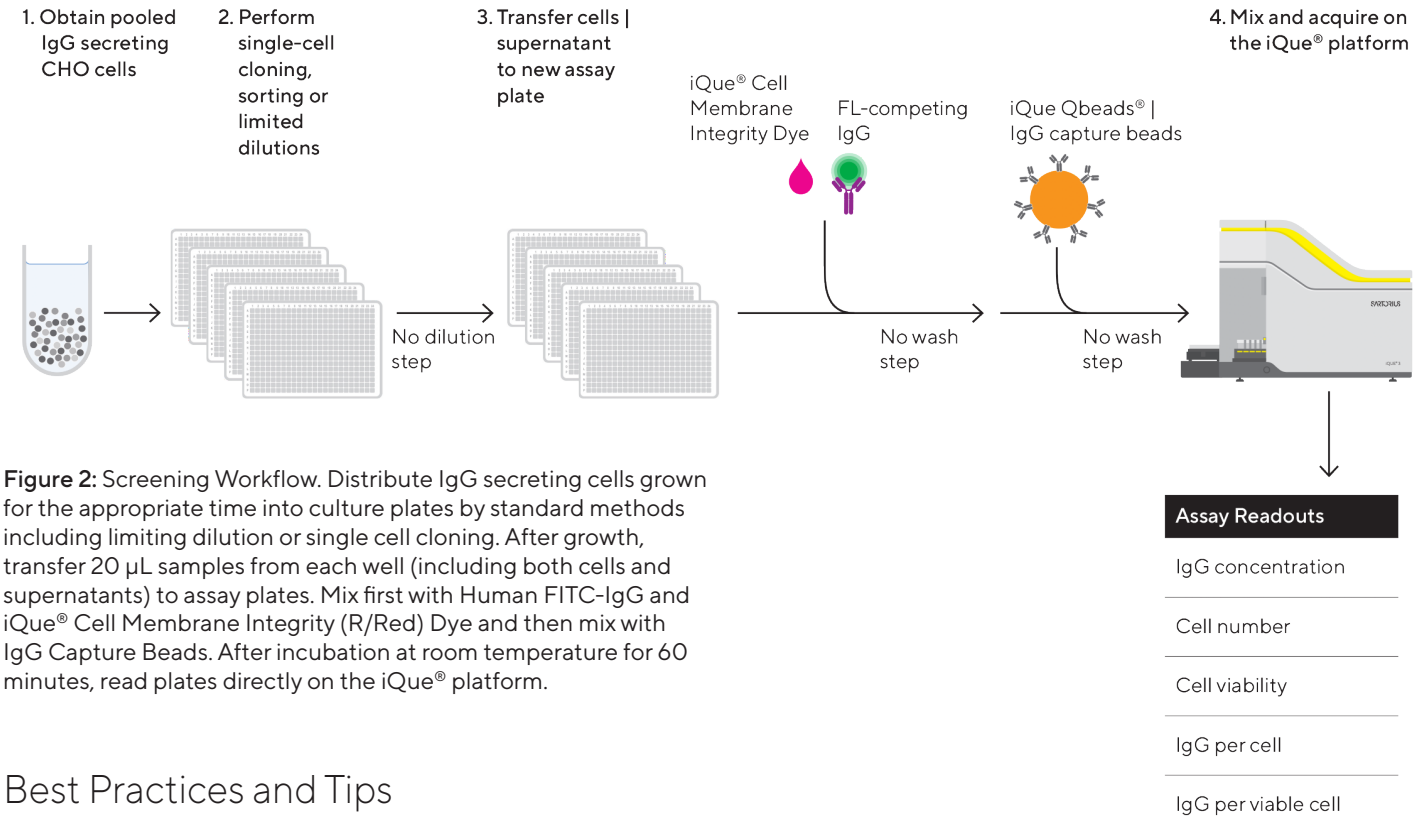


Figure 2: Screening Workflow. Distribute IgG secreting cells grown for the appropriate time into culture plates by standard methods including limiting dilution or single cell cloning. After growth, transfer 20 µL samples from each well (including both cells and supernatants) to assay plates. Mix first with Human FITC-IgG and iQue® Cell Membrane Integrity (R/Red) Dye and then mix with IgG Capture Beads. After incubation at room temperature for 60 minutes, read plates directly on the iQue® platform.

Best Practices and Tips

Running the Assay in 96-well Format

The assay protocol described in this manual is designed for 384-well plate format. To perform the assay in 96-well plates, use the same protocol and volumes designed for a 384-well format but adjusted to a 96-well format according to the volume table provided in **Pages 3–4**. iQue® recommends the use of 96-well bottom plates (iQue®, Cat. No. 90151). This assay kit provides iQue Forecyt® templates for both 384-well and 96-well formats.

Manual Pipetting Recommendation

This protocol requires pipetting 5 µL volumes of liquid. If pipetting manually instead of with an automatic liquid handler, be careful during the 5 µL volume transfer of the prepared reagent from the reservoir to the assay well. If the plate is empty, touch the tip to the well bottom and then release all the liquid to transfer 5 µL volume into the well. If the plate already has a reagent or sample in the wells, touch the pipette tip to the upper inner wall of the well at 45-degree angle before releasing the 5 µL prepared reagent. Touching the wall of the well prevents the 5 µL liquid droplet from hanging on the pipette tip instead of releasing into the assay well. A five second spin in a plate centrifuge will force the prepared detection reagent to the well bottom to mix with the existing reagent | sample already in the well.

Shaking

This assay requires shaking the micro-titer plate to quickly mix the sample | reagents. To use the shaker on the iQue® platform. (1) Click on Device in the menu bar. (2) Scroll down to Manual Control. (3) In the Manual Control window, use the arrows to set the RPM to 2000. (4) Once the On button is selected, the shaker will begin to shake and continue to until the On button is unselected.

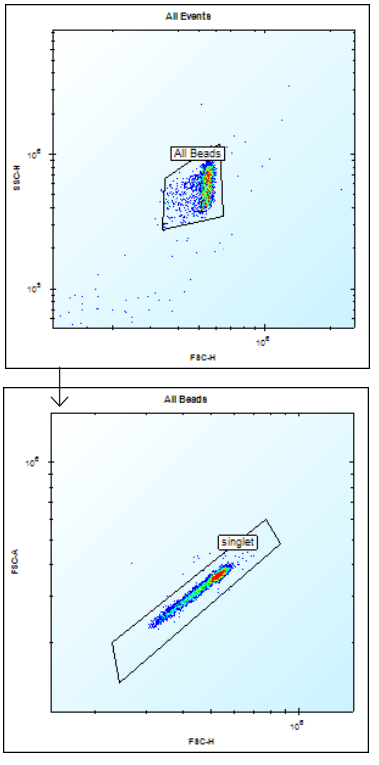


Figure 3.
Steps for using the shaker on the iQue® platform.

Before Beginning

- Briefly centrifuge all vials before use to prevent reagent loss.
- Mix the dye with pipette or briefly vortex prior to use.
- Vigorously vortex capture beads prior to use to ensure a homogenous solution throughout the procedure. Beads tend to settle and aggregate over time.

Reagent Preparation

1.0 Mix Sample Reaction Buffer with BSA

1.1 Add the entire volume of Sample Reaction Buffer solution to the lyophilized bovine serum albumin (BSA) bottle. Label the BSA bottle "Sample Reaction Buffer with BSA".

1.2 Gently mix by inverting the bottle multiple times until the BSA has completely dissolved. Let the bottle sit at room temperature until you are ready to use it. If there are still particles, gently mix again before use. BSA does not affect IgG quantification.

2.0 Do Serial Dilution: Preparation of Control Human IgG Protein Standard

For ranking studies or assays where absolute concentrations are not required, the standard curve generation step may be omitted; results can be reported as relative amounts of IgG with inverse relationship with median fluorescence intensity (MFI) of the capture bead. If an IgG standard is required, it is best to use the IgG isotype purified from the same culture sample or an IgG isotype similar to IgG in your unknown samples. For example, if your unknown samples have IgG4κ, use the same IgG4κ for your standard. With different IgG isotypes there may be a slight difference in binding to the capture beads used in this assay. iQue® provides 1 vial of control human IgG protein standard (10 mg/mL stock concentration, IgG isotype mixture) for standard curve generation to quantify the IgG sample. To use your own IgG as standard, perform a side-by-side comparison test with the iQue® IgG standard to confirm your own standard is working in the assay before running a screening. Your IgG isotype-specific standard curve may be similar to or slightly different from the iQue® IgG (mixture) standard curve, depending on the IgG isotype. This is normal and will not affect your IgG quantification.

When preparing iQue® IgG standards for quantitative protocols, using larger transfer volumes reduces the effects of pipetting error. Generally, we recommend titrating standards in at least 500 µL volumes. Discard excess standard. The following protocol is a guideline you can adapt or optimize for your laboratory.

2.1 For a 384-well plate, prepare 16 micro-tubes. Label them #1–16. Follow the same protocol for a 96-well plate but perform a 12-point dilution. (Alternatively, use microcentrifuge tubes or 16 x 75 mm FACS tubes or 12-channel pipette reservoirs.)

2.2 To tubes # 2–16, add 500 µL of fresh cell culture media. This is the same media used to grow your sample culture. Set aside after adding the cell culture media, .

2.3 To tube #1, add 900 µL of fresh cell culture media. Then add 100 µL of the provided Control Human IgG to the media in tube #1. The mixture should total 1 mL. Mix well by pipetting up and down six times. This sample is the highest concentration sample (1,000 µg/mL) for the standard curve (top standard).

The control Human IgG Protein Standard is provided as a solution at 10 mg/mL concentration. Following these instructions will yield a top concentration in the assay of 1,000 µg/mL. This also constitutes the highest point in the standard curve. For a higher top concentration for standard because the IgG sample is higher than 1,000 µg/mL IgG, adjust the dilution factor and | or the volume of cell culture media used in each tube. If a small volume of culture media is being used for standard dilution, be very careful handling the liquid. If this assay is used for media optimization purposes, use the sample reaction buffer plus BSA to do a serial dilution of the standard.

2.4 From tube #1, remove 500 µL of standard, and transfer to tube #2. Gently pipet up and down at least 6 times to completely mix the solutions.

2.5 From tube #2, transfer 500 µL to tube #3 and mix by pipetting. Continue transferring and mixing through tube #15. Do not transfer any standard into tube #16. Tube 16 will be the media only negative control.

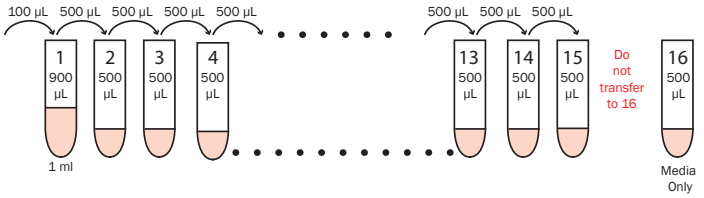


Figure 4: For 384-well Plate: 16-point Serial Dilution of IgG Standard.

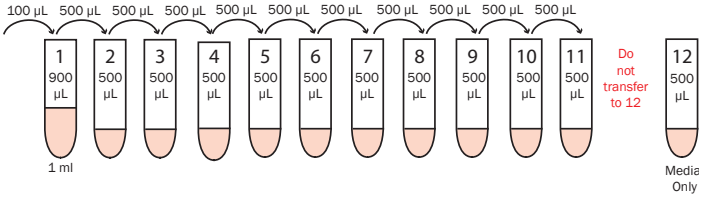


Figure 5: For 96-well Plate: 12-point Serial Dilution of IgG Standard

2.6 Add the prepared standards to the assay plate. Transfer 20 µL of each standard to the appropriate wells of the plate. (See a representative plate design for standard wells in **Figure 5**.) After transfer, cover the assay plate with a lid to prevent evaporation. There will be excess standard remaining. Use it on the same day, or discard. Do not save diluted standards.

3.0 Mix 2 Dyes:Prepare Detection Reagents (1st Reservoir)

For 1 x 384-well kit, label a reservoir "Detection Reagent."

3.1 To the Detection Reagent reservoir, add 2.5 mL of the Sample Reaction Buffer with BSA.

3.2 Add 25 µL of Human FITC-IgG dye to the Detection Reagent reservoir.

3.3 Add 12.5 µL of iQue® Cell Membrane Integrity (R/Red) Dye to the Detection Reagent reservoir.

3.4 Mix the dye reagents in the buffer by manual pipetting. Cover reservoir with foil to prevent evaporation and protect from light. DO NOT transfer the mixed dye to the assay plate.

For other kit sizes, please refer to **Table 1** to prepare the reagents. For the largest size kit, use a bottle of an appropriate size to prepare the detection reagent.

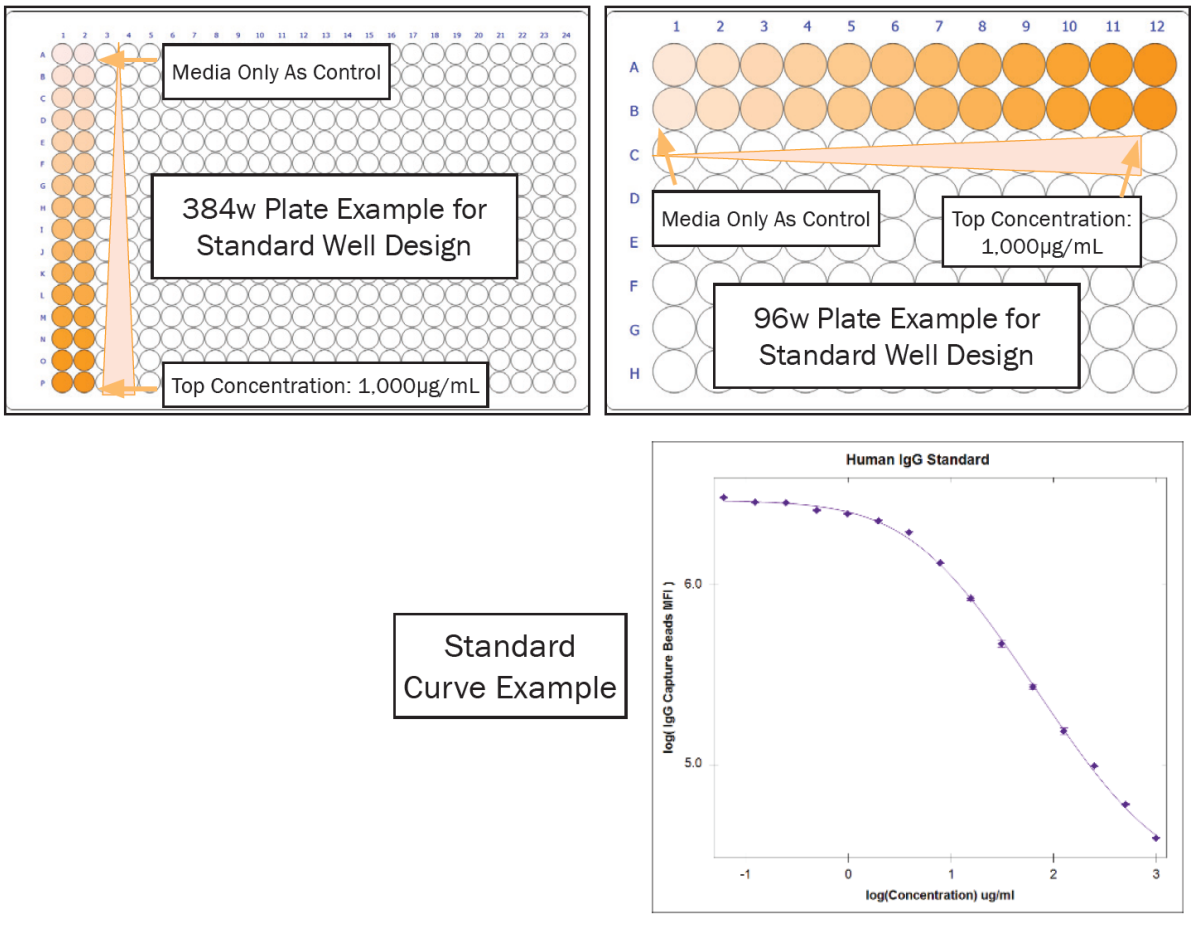


Figure 6: Example design of the IgG Standard wells in 384-well plate and 96-well plate. We have provided a representative standard curve with 1:2 serial dilution with the top concentration at 1,000 µg/mL.

Format (wells)	Sample Reaction Buffer with BSA	Human FITC-IgG (1:100 dilution)	iQue® Cell Membrane Integrity (R/Red) Dye (1:200 dilution)
1 x 384	2.5 mL	25 µL	12.5 µL
5 x 384	12.5 mL	125 µL	62.5 µL
20x 384	50 mL	500 µL	250 µL
50 x 384	125 mL	1250 mL	625 µL

Table 1: These volumes will create enough prepared dye to add 5 µL per well for a full plate with minimal overage. To prepare stain for partial plates, or, for more overage, dilute the reagents at the dilution factors specified in Sample Reaction Buffer to the desired total volume.

4.0 Dilute IgG Capture Beads (2nd Reservoir)

Label a 50 mL reservoir "Capture Beads" and follow these steps for 1 x 384 well assay:

4.1 Add 2.3 mL of the sample reaction buffer with BSA to the reservoir.

2.2 Vortex the stock IgG capture beads for 10 seconds.

2.3 Transfer 160 µL of the IgG capture beads to the sample reaction buffer in the capture beads reservoir.

2.4 Mix the beads in the buffer by manual pipetting. Cover reservoir with foil to prevent evaporation and protect from light. DO NOT transfer the prepared capture beads to the assay plate yet.

For other kit sizes, refer to the following table to prepare the reagents. To run a 96-well plate format, use a 384-well kit to run four 96-well plates or directly use 1 x 96-well kit.

Format	Sample Reaction Buffer with BSA	Stock IgG Capture Beads (1:15 dilution)
1 x 384 wells	2.3 mL	160 µL
5 x 384 wells	11.2 mL	800 µL
20 x 384 wells	49 mL	3.5 mL
50 x 384 wells	124 mL	8.8 mL

Table 2: Reagent Preparation for Sample Reaction Buffer and IgG Capture Beads.

Note: These volumes will create enough prepared dye to add 5 µL per well for a full plate with minimal overage. To prepare stain for partial plates, or, for more overage, dilute the reagents at the dilution factors specified in sample reaction buffer to the desired total volume.

Assay Set Up

Total Protocol Time: 60 minutes
Total Hands-On Time: Approximately 10 minutes

This assay uses a no-wash workflow and provides results in terms of IgG concentration (e.g., µg/mL).

Note: This is a competition assay. The sequential steps in the assay set up instructions below are critical to the success of the experiment. After reagent preparation, the assay components must be added in this exact order:

1. IgG Sample | IgG Standard
2. Detection Reagent Mixture (Human FITC-IgG and iQue® Cell Membrane Integrity (R/Red Dye)
3. Capture Beads

5.0 Add IgG Sample | IgG Standard

5.1 Add 20 µL of IgG sample (either IgG supernatant, or IgG supernatant | cell mixture) to each assigned well of a 384-well plate.

Warning: If the standard prepared earlier was not added in, it is time to transfer 20 µL IgG standard to each well designated for IgG standards.

If the IgG | cell mixture is used as a sample, make sure to mix cells in the original source plate or flask to keep cells in the suspension before completing the transfer.

6.0 Add Detection Reagent Mixture (Human FITC-IgG and iQue® Cell Membrane Integrity (R/Red) Dye)

6.1 Add 5 µL of prepared detection reagent (the mixture of Human FITC-IgG dye and iQue® Cell Membrane Integrity (R/Red) Dye prepared earlier). Do a quick spin of the plate (500 x g, 5 seconds) to ensure that samples are at the bottom of the wells and not attached to the sides.

6.2 Shake the plate to ensure thorough mixing. Use the shaker on the iQue® platform and mix for 20 seconds at 2,000 RPM. (Refer to **Best Practices and Tips** for how to do 5 µL manual pipetting and how to use the shaker for mixing)

7.0 Add the Prepared Capture Beads Mixture

7.1 Briefly mix the pre-diluted capture beads in the capture beads reservoir by gentle pipetting to keep the beads in suspension. Add 5 µL of the pre-diluted capture beads mixture to each well. Mix the beads in the reservoir 1–2 times while preparing a full assay plate to prevent beads from precipitating to the reservoir | tube bottom.

7.2 Do a quick spin of the plate (500 x g, 5 seconds) to ensure that samples are at the bottom of the wells and not attached to the sides.

7.3 Mix the plate to ensure thorough mixing. Use the shaker on the iQue® platform and mix for 20 seconds at 2,000 RPM. (Refer to **Best Practices and Tips** for how to do 5 µL manual pipetting and how to use shaker for mixing).

7.4 Incubate the plate at room temperature for 1 hour, covered with a lid and protected from light.

7.5 After incubation is complete, acquire data on the iQue® platform.

Note: iQue® recommends using iQue® QSol buffer in the buffer station (the first rinse station) for tubing priming.

Data Acquisition and Analysis

8.0 Launch iQue Forecyt®.

9.0 Create a new experiment using the pre-defined assay template provided with your kit.

The template contains a preset sampling protocol for data acquisition. The protocol may be changed to optimize the sampling for a specific experiment.

10.0 The following guidance defines recommended acquisition settings in the Protocol tab in iQue Forecyt®.

- Pre-Plate Prime: 60 seconds. Prior to sampling a plate, the system will prime the tubing with the iQue® QSol buffer.
- Pre-Plate Shake: 15 seconds at 2,400 RPM. Prior to sampling a plate, the shaker will agitate the samples at the specified speed and time. The specified shake speed assumes that the sample contains 30 µL of total sample volume. For samples that contain different volumes, see **Appendix C**.
- Sampling Order: By row, column, zig-zag.
- Sip Time: 1 second. Sip time is proportional to the sample volume collected per well. Sip time can be increased to acquire more events per well. The tradeoff is slightly slower overall read- time.
- Additional Up Time: 0.5 seconds. Up Time specifies the amount of time the probe pauses before moving to the next well and determines the spacing between samples. We recommend no adjustment.
- Pump Speed: Standard speed (15 RPM on iQue® Standard, 29 RPM on iQue® Screener Plus and iQue® 3). This specifies the rate at which samples pass by the detectors. The default setting of the standard roughly corresponds to introduction of ~1.5 µL per second. We recommend no adjustment. We recommend no adjustment.
- Interwell Shake: 4 seconds at 2,400 RPM after every 12 wells if sampling by row or after every 16 wells if sampling by column. Interwell Shake inserts a rinse and shake after a specified number of wells to keep sample particles in suspension. The frequency of Interwell Shake can be adjusted after any number of wells to avoid sample particles precipitating to the well bottom.
- Speed (for iQue® or HTFC): Medium. Specify the flow rate of the detector. This affects the resolution of the data. We recommend no adjustment.
- Threshold: FSC-H at 80,000 on iQue® Standard; 100,000 on iQue® Screener Plus and iQue® 3. Specify the lower thresholds for data acquisition. The current setting on FSC will filter out events with size below 2 microns. We recommend no adjustment.

11.0 After making any Protocol adjustments, click Run on the Controller to acquire data.

12.0 During the plate read, data will automatically begin populating into the pre-defined analysis

template.

13.0 Verify that the sample data aligns with the pre-defined gating strategy from the template. If necessary, adjust the gates in each plot to encompass the proper bead populations. All gates can be moved by clicking the gate label and dragging to the desired location.

13.1 **Identify Cell and Bead Populations.**

If necessary, move the "cells" gate and "beads" gate to encompass all regions of interest. While the iQue Forecyt® template will gate the cells and beads automatically, the gate may need manual adjustment to include the smaller bead population. The smaller bead population must be included in the gate of all beads. The size of these gates may be enlarged to include additional cell populations in the analysis.

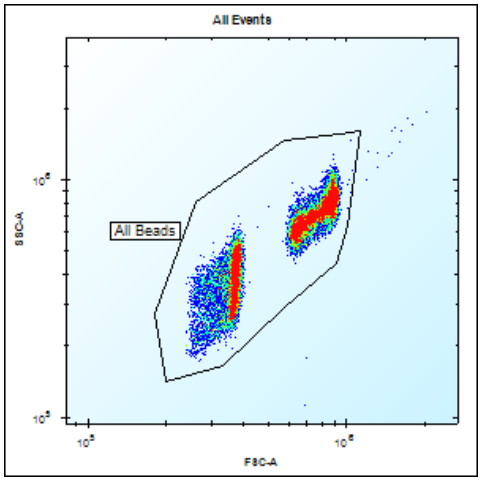


Figure 7: Gate Beads and Cells.

13.2 **Identify Singlet Beads.**

Analyzing only the singlet bead population helps avoid analysis artifacts created when analyzing aggregates of beads. The singlet population is on the ~45° angle on the FSC-H vs FSC-A plot.

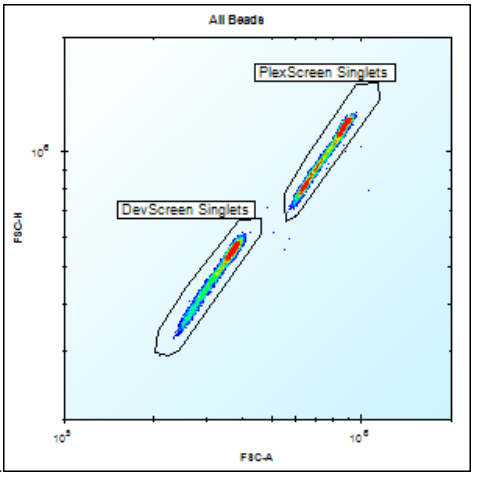


Figure 8: Singlet bead gate.

13.3 **Identify Viable Cells.**

DNA dye binding to the cells gives a measurement of cell viability in the FL4-H (iQue® Screener Standard) or RL1-H (iQue® Screener PLUS and iQue® 3) histogram. Positive cells (right peaks) represent the non-viable population. Adjust the gate as necessary to encompass the left-most peak. This represents the viable cells that excluded the dye. This gate will be used to report the percentage of viable cells in each well.

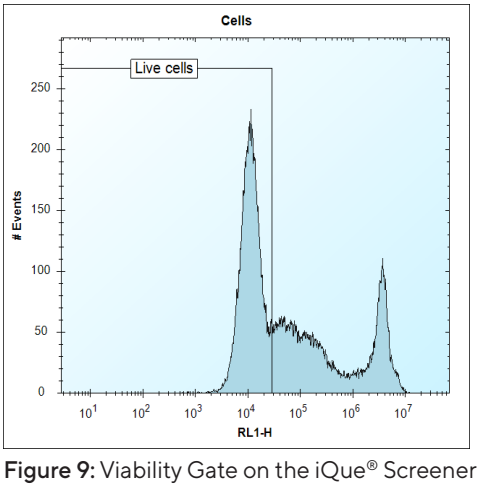
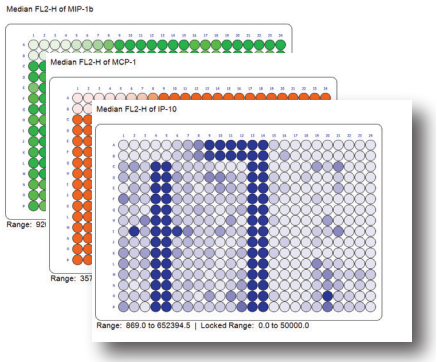


Figure 9: Viability Gate on the iQue® Screener PLUS

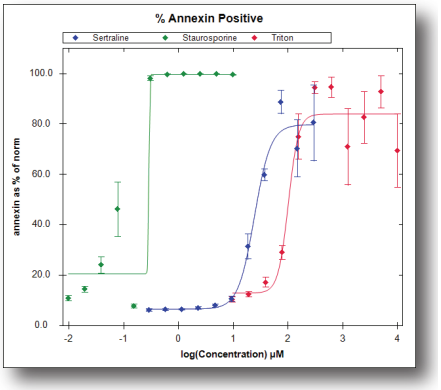
Visualization of Screening Results

After all the gates have been verified and adjusted as necessary for the plate-level data set, you can generate additional analyses including heat maps, dose responses, and standard curves (examples below).

Heat Maps

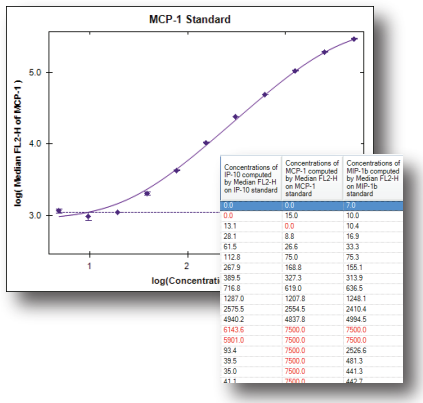


Dose Response Curves

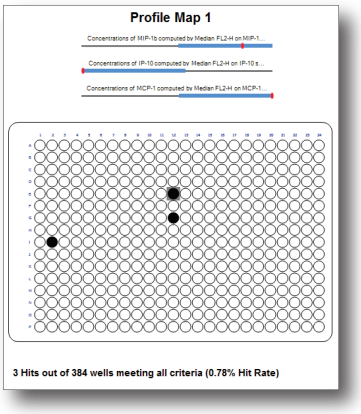


PDF Data Reports

Standard Curves



Profile Maps



Overlay Plots

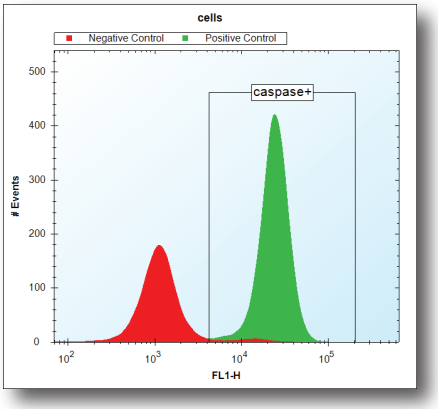


Figure 10. Data visualization examples.

Appendix A: FAQ

Q1: Why does the standard curve look like a straight flat line at saturation level instead of an S curve?

Answer: The competition setup is wrong. You must mix the unlabeled standard IgG with human FITC-IgG first. Add the capture beads at the end. You must strictly follow the competition setup protocol to run this assay.

Q2: Why does the quantified IgG concentration from the iQue® Human IgG Titer & Viability Kit assay seem slightly different from my traditional IgG quantitation assay?

Answer: The IgG standard provided by iQue® is a purified natural IgG protein with all isotypes (IgG1-4). Different IgG isotypes have slightly different binding to capture beads. The IgG standard with the same isotype as IgG in the unknown sample may get better IgG quantitation for your unknown sample. For example, if human IgG in your unknown sample is IgG4κ, human IgG4κ may be a better standard than the iQue® IgG (mixture). Run a side-by-side comparison test to confirm your IgG standard works. In addition, please make sure your unknown IgG samples are within the linear range of the IgG standard curve. Samples out of the linear range will cause more variation. For very precise IgG concentration you may need to dilute your sample so it falls within the linear range of the standard curve.

Q3: I have bead number variation in the assay plate. Does bead number variation decrease my assay performance?

Answer: Bead variation generally does NOT affect assay performance, as long as you have acquired a hundred or more beads per well. The bead number variation does NOT change the detection median fluorescence intensity (MFI) of the capture beads. The IgG quantification is based on bead detection MFI. You can avoid bead number variation by mixing the diluted beads in reservoir periodically during the manual transferring of prepared beads to assay plate.

Q4: I have difficulty in precisely pipetting 5 µL volume to the assay plate. Is there a better way to handle transferring a 5 µL volume from in front of reservoir to assay plate?

Answer: Automated liquid handlers can generally pipette 5 µL volume very precisely. If you do not use an automated liquid handler, we recommend a 12-channel pipette (5-120 µL) for the liquid transfer (See Appendix D). The pipette tip must touch the wall of the well at 45-degree angle before you manually release the 5 µL prepared reagent. Touching the wall of the well prevents the 5 µL liquid droplet from hanging on the pipette tip instead of releasing into the assay well. A quick spin will force the prepared detection reagent on the wall to the well bottom to mix with other assay components already existing in the well.

Q5: My culture plate is a 96-well format. Can this 384-well assay kit be adapted to 96-well format?

Answer: Yes. A single 384-well assay kit can be used for 4 plates assay in a 96-well format. If performing the assay in 96-well plates, you may use the same protocol and protocol volumes designed for a 384-well format. iQue® recommends the use of 96-well V-bottom plates (iQue®, Cat. No. 90151). You may need to adjust shake speeds and other protocol settings to accommodate the 96-well plate format. The 384-well assay kit provides assay templates for both 384-well format and 96-well format on the USB drive included in the kit package.

Q6: I run CHO cell samples through iQue® platform. Sometimes it clogs the system. How do I avoid the clogging issue?

Answer: Follow the cleaning and maintenance instructions in the iQue® Screener manual to prevent clogging issues. Make sure to use iQue® QSol cartridge on buffer station (1st rinse station) for tubing priming. In addition, do not use your own adhesive seal to seal the assay plate or the cartridge on any rinse station. Adhesive seals may block/clog the sampling probe or even the cytometer engine if the sampling probe touches your adhesive seal during the sampling process. If your cell samples do cause the system clogging, change the sampling tubing and fluidic link. Clicking Clean on the Controller will bring up the Clean Wizard which will walk you through the cleaning steps. Normally the clogging will go away. If not, repeat the long clean and unclogging processes.

Q7: What is the detection limit of my CHO cell density in iQue® Human IgG Titer & Viability Kit assay?

Answer: Normally you can linearly quantify the cell events when the CHO cell density is between 0.01 million per mL to 10 million per mL. You can increase the sip time per well to increase the number of cells acquired and decrease the variation when the cell density is very low. Optimizing the sip time can help you achieve your specified statistical significance.

Q8: Does the iQue® Human IgG Titer & Viability Kit assay work with mouse IgG quantification?

Answer: The iQue® Human IgG Titer & Viability Kit assay is validated to quantify human IgG, not mouse IgG. Although the iQue® Human IgG Titer & Viability Kit assay may detect mouse IgG or rank mouse IgG in relative quantity, it may have a compromised performance, and the quantification may not be precise.

Q9: Does iQue® Human IgG Titer & Viability Kit assay work with the Ig-like protein or Fc-fusion protein?

Answer: The iQue® Human IgG Titer & Viability Kit assay is only validated to quantify human IgG. It may detect and quantify IgG Fc fragment and/or Fc-fusion protein, however, the signal and assay performance may decrease. We recommend you to do further optimization. The iQue® Human IgG Titer & Viability Kit assay may not detect or quantify Ig-like protein at all.

Q10: Can I multiplex the iQue® Human IgG Titer & Viability Kit assay with measurements of other cell health or IgG endpoints?

Answer: Yes, it is possible. You may multiplex this assay with these measurements: apoptosis (iQue® Human 4-Plex Apoptosis Kit); autophagy measurement; IgG light chain measurement; or antigen binding measurement. However, you may need to do extensive optimization to confirm assay performance in your biology.

Appendix B: Mixing Samples with the iQue® Shaker

Plate Type	Well Volume	Max. RPM
96-Well	20–40 µL	2600
96-Well	40–60 µL	2200
96-Well	60+ µL	A | O*
384-Well	10–30 µL	3000
384-Well	30–50 µL	2800
384-Well	50+ µL	A | O*

Table 4: iQue® and iQue® Screener PLUS platforms

Plate Type	Well Volume	Max. RPM
96-Well	20–40 µL	2800
96-Well	40–60 µL	2400
96-Well	60+ µL	A | O*
384-Well	10–30 µL	3500
384-Well	30–50 µL	3000
384-Well	50+ µL	A | O*

Table 5: HTFC Screening System

Plate Type	Well Volume	Max. RPM
96-Well	20–40 µL	3200
96-Well	40–60 µL	2400
96-Well	60+ µL	A | O*
384-Well	10–30 µL	3500
384-Well	30–50 µL	3100
384-Well	50+ µL	A | O*
1536-Well	up to 6 µL	5000

Table 6: iQue® Screener HD platform

*A | O = Additional Optimization necessary. While it is possible to run these volumes, they are not routinely tested by the assay development team. To determine ideal shake speeds for high volume assays, iQue® recommends starting at low RPM values and slowly increasing to higher values.

Appendix C: Plate-type Recommendations and Automated Wash Protocols for Microplates

The following plate types and aspiration settings have been extensively tested with the iQue® assay products.

Plate Type	Well Type	Manufacturer	Manufacturer Product
384-well	V-bottom	Greiner®	781280
96-well	V-bottom	iQue®	10149

Table 7: Plate Type Recommendations

When using the above plate types, the following aspiration programs have been tested on a BioTek® ELx405 Select. If using a different plate washer brand or model, it is possible to approximate the aspiration settings on a different system.

iQue® highly recommends that wash protocols use an automated plate washer. Manual aspiration of plates and | or plate inversion techniques could result in severe sample loss.

Plate Type	Height Setting	Height Offset	Rate Setting	Aspiration Rate
384-well, V-bottom	#31	3.937 mm	#6	15 mm/sec
96-well, V-bottom	#40	5.08 mm	#6	15 mm/sec

Table 8: Aspiration Recommendations

Appendix D: Liquid Handler Recommendations

iQue® recommends the following liquid handlers:

12-channel pipette for manual transfer of liquid to the plate:

- Manual 12-channel pipette mLINE or Tacta, 5–100 µL (Sartorius®);
- Electronic 12-channel pipette eLINE or Picus, 5–120 µL (Sartorius®).

Automated liquid handler:

- Personal Pipettor, 96- or 384-channels (Apricot Designs®);
- MicroFlow Select, 8 channels (BioTek®).

Single-channel pipette for reagent preparation:

- Manual single-channel pipette mLINE or Tacta (Sartorius®);
- Electronic single-channel pipette eLine or Picus (Sartorius®).

Sales and Service Contacts

For further contacts, visit www.sartorius.com

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Publication No. #11317 Rev H.

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