SARTORIUS

Product Guide

iQue® Mouse IgG Type and Titer Kit

Product Information

Notice to Purchaser

The iQue® Mouse IgG Type and Titer Kit is a member of the iQue® product line that has been tested extensively 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.

This product is manufactured and sold by Sartorius for research use only. The kit and components are not intended for diagnostic or therapeutic use. Purchase of the product does not include any right or license to use, develop, or otherwise exploit this product commercially. Any commercial use, development or exploitation of this product without the express written authorization of Sartorius is strictly prohibited. Not for resale.

List of Catalog Numbers

Product Name	Format	Cat. No.
iQue® Mouse IgG Type and Titer Kit	1 x 384 wells	91165
iQue® Mouse IgG Type and Titer Kit	5 x 384 wells	91166
iQue® Mouse IgG Type and Titer Kit	1 x 96 wells	91168

Table 1: List of Catalog Numbers

Kit Contents

Product Name	Format
Mouse IgG Capture Beads	1 vial
Mouse FITC-IgG	1 vial
Mouse IgG Standard	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 LP		B/Red			
675/25				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/ Crim-son	

Table 4. iQue® PLUS Detector Channels

Note: For iQue® PLUS with VYB lasers, the bead detection channel is B/Green and the cell viability detection channel is Y/Red. For iQue® PLUS with BR lasers, the bead detection channel and the cell viability detection channel are the same as iQue® 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
- 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
- Fresh complete cell culture media (Same media used to grow sample cell culture)
- Microcentrifuge tubes and | or 15 mL conical tubes
- Two 50 mL reagent reservoirs (Example source: VWR®, Cat. No. 89094- 680) for reagent preparation purposes
- 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), optional for preparing serial dilutions
- Appropriate liquid handler or multi-channel pipette (Example Source: BioHit®, Cat. No. LH745441, Picus NxT, 12channel, 5 to 120 µl; **Appendix E**)

Table of Contents

Background

The iQue® Mouse IgG Type and Titer Kit was designed for ease of use in multiplexing. It provides a straightforward no-wash, no-sample dilution workflow that doesn't compromise assay performance and offers these unique advantages:

- Precision — Multiplexed isotype-capture bead assay enables more precise quantitation of IgG for each isotype.
- Simultaneous isotyping measurement — Provides clone purity information when two or more isotypes are present in the same well, and determines the DNA primer set for downstream gene cloning.
- Simultaneous measurement of cell count and cell viability — Monitors cell proliferation and cell health in the original cell cloning plates.
- No-sample dilution assay — Wide dynamic range (0.05 µg/mL to 50 µg/mL) enables transfer of cell culture samples directly into assay plates without a dilution step.

Assay Principles

The iQue® Mouse IgG Type and Titer Kit is a no-wash assay that enables the simultaneous measurement of mouse IgG isotypes, the mouse IgG quantity for each isotype, cell number and cell viability from each well of the screening plates. This is a competition assay in which fluorescently-labeled mouse IgG (mouse FITC-IgG) is added to mouse samples containing secreted IgG from hybridomas or B-cell cultures. The mouse FITC-IgG and non-labeled mouse sample IgG compete for binding to IgG capture beads in an isotype-specific manner. The amount of IgG isotype present in the sample is inversely proportional to the isotype-specific bead-associated fluorescence. Signals across four different isotype-specific beads determine the IgG isotype in the assay well. 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 allow entry of the dye into the intracellular space where it then localizes in the nucleus and binds to DNA by intercalation.

Quantitative readouts from this assay can be measured as fluorescence intensity or extrapolated to a concentration (µg/mL) in solution via the use of isotype-specific standard curve.

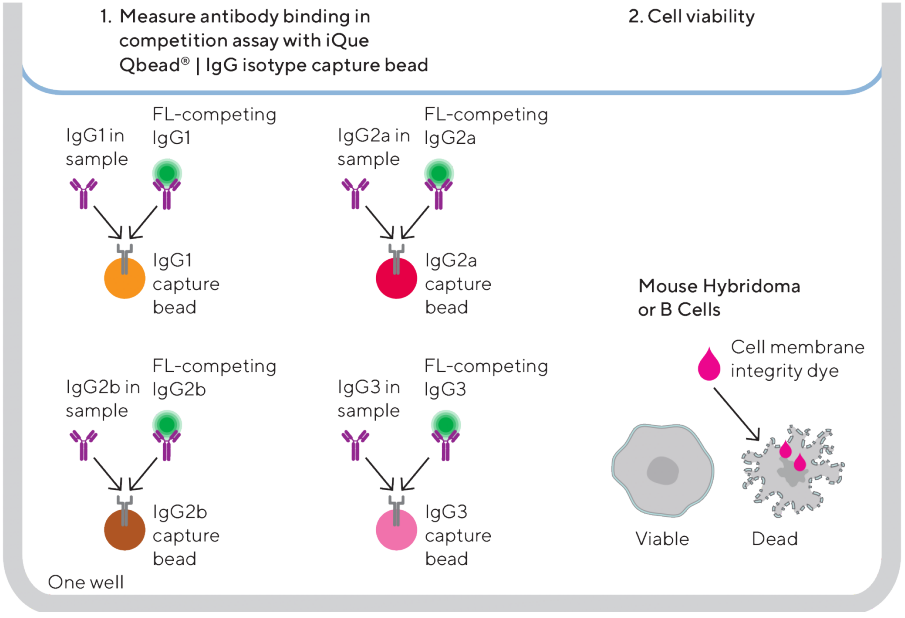


Figure 1: iQue® Mouse IgG Type and Titer Kit assay principles. The no-wash competition assay functions on the differential binding of cell-secreted IgG vs mouse FITC-IgG to IgG Capture Beads in an isotype- specific manner. Samples with high IgG concentrations will exhibit a low fluorescence signal, whereas the absence or low concentration of solution IgG would result in a large fluorescence signal. The assay dynamic range is between 0.05 µg/mL to 50 µg/L.

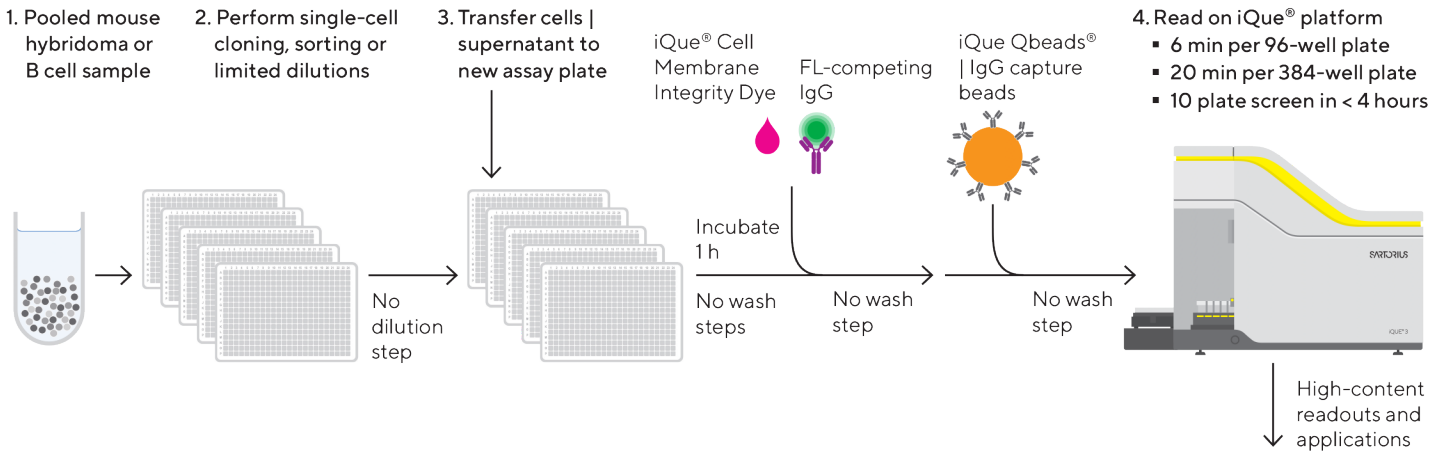


Figure 2: Screening Workflow. Distribute mouse hybridoma fusion cells (grown for the appropriate time) into culture plates by either limiting dilution or single cell cloning or single cell sorting. After growth, transfer 20 µL samples from each well (supernatant only or supernatant plus cells) to assay plates. Mix first with mouse FITC-IgG and iQue® Cell Membrane Integrity (R/Red) Dye in the assay plates, and then mix with mouse IgG capture beads. After incubation at room temperature for 60 minutes, read plates directly on the iQue® platform.

Readouts	Applications
IgG isotypes	▪ Isotyping: 1, 2a, 2b, 3 or mixture ▪ Identify pure monoclonal ▪ Determine DNA primers for gene cloning
IgG isotype concentration	▪ Normalize concentrations for downstream functional assays
Total IgG concentration	▪ Confirm hybridoma | B cell stability with antibody secretion
Cell count and health	▪ Monitor hybridoma | B cell proliferation and health in original cell cloning plates

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 (for Quick Mixing)

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.

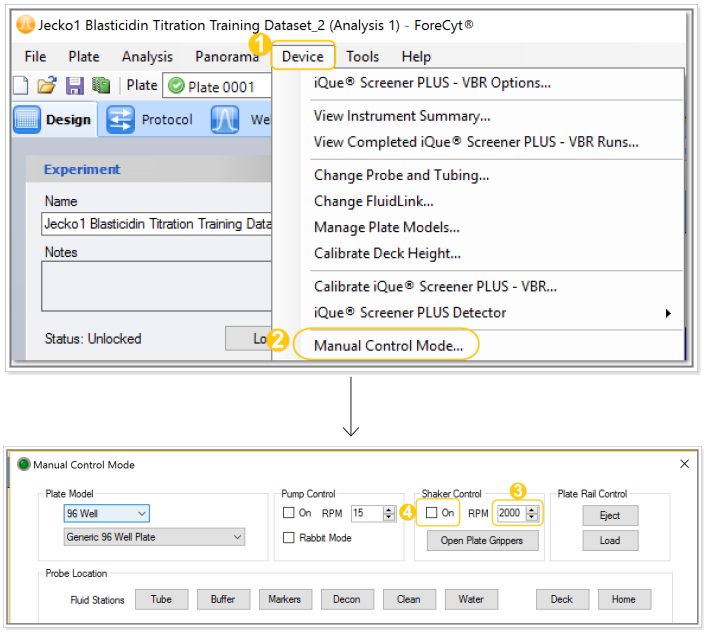
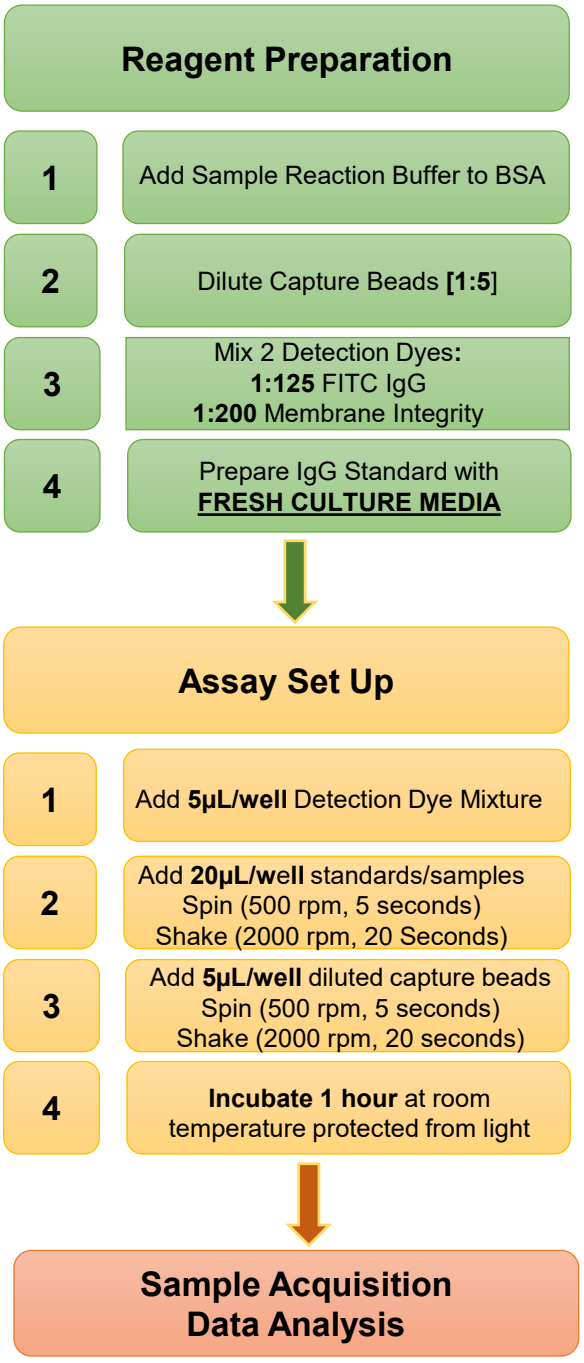


Figure 3. Steps for using the shaker on the iQue® platform.

Assay Protocol Overview

This protocol is designed to measure mouse IgG quantity and isotype in mouse hybridoma samples containing 1–50 µg/mL IgG. To measure mouse IgG and isotype in mouse primary B cell samples containing less than 1 µg/mL IgG (0.1–2 µg/mL IgG), refer to **Appendix A** for instructions for a high sensitivity assay. The following diagram is an overview of the protocol to help you plan your work. Detailed protocol instructions are provided in the next section.



Before Beginning

- Briefly centrifuge all vials before use to prevent reagent loss.
- Mix the dye with pipette or briefly vortex prior to use.
- 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 ready to use. If there are still particles, gently mix again before use. BSA does not affect mouse IgG quantification.

2.0 Dilute Mouse IgG Capture Beads (1st Reservoir)

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

2.1 Add 1.92 mL of the Sample Reaction Buffer with BSA prepared earlier to the reservoir.

2.2 Vortex the Mouse IgG Capture Beads vial from the kit for 30 seconds.

2.3 Transfer 480 µL of the Mouse IgG Capture Beads to the Sample Reaction Buffer in the Capture Beads reservoir.

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

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	Mouse IgG Capture Beads (1:5 dilution)
1 x 384 wells	1.92 mL	480 µL
5 x 384 wells	9.6 mL	2.4 mL
1 x 96 wells	0.48 mL	120 µL

Table 5: Reagent Preparation for Sample Reaction Buffer and Mouse IgG Capture Beads

The volumes defined above 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.

3.0 Mix 2 Detection Dyes (2nd Reservoir)

Label the second 50 mL reservoir "Detection Reagent." For 1 x 384-well assay add two dyes, Mouse FITC-IgG and iQue® Cell Membrane Integrity (R/Red) Dye dye.

3.1 To the Detection Reagent reservoir, add 2.4 mL of the Sample Reaction Buffer with BSA.

3.2 Add 19.2 µL of Mouse FITC-IgG to the Sample Reaction Buffer with BSA in the Detection Reagent reservoir.

3.3 Add 12 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 prepared mixed dye to the assay plate yet.

For other kit sizes, please 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. The dilution formula for a 1 x 96 wells plate is also provided in the table.

Format (wells)	Sample Reaction Buffer with BSA	Mouse FITC-IgG (1:125 dilution)	iQue® Cell Membrane Integrity (R/Red) Dye (1:200 dilution)
1 x 384	2.4 mL	19.2 µL	12 µL
5 x 384	12 mL	96 µL	60 µL
1 x 96	0.6 mL	4.8 µL	3 µL

Table 6: Reagent Preparation for Sample Reaction Buffer, Mouse FITC-IgG, and iQue® Cell Membrane Integrity (R/Red) Dye

The volumes defined above 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 to increase overage, calculate the total volume required and dilute the Mouse FITC-IgG 1:125 and iQue® Cell Membrane Integrity (R/Red) Dye 1:200.

4.0 Prepare Mouse IgG Standard

The kit contains one vial of Mouse IgG Standard with 4 mouse IgG isotype proteins (a mixture of IgG1, 2a, 2b and 3 at a concentration of 200 µg/mL for each isotype). The Mouse IgG Standard is used to generate a standard curve between 0.05 µg/mL to 50 µg /mL for each isotype.

The following dilution protocol is a guideline that can be adapted or optimized for your laboratory.

4.1 For a 384-well plate, prepare 16 micro-tubes. Label them #1–16. (An empty 96-well plate or 12-channel pipette reservoir can also be used to dilute the standard.)

4.2 To tubes #2–16, add 50 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.

4.3 To tube #1, add 75 µL of fresh cell culture media. Then add 25 µL of the stock mouse IgG standard from the kit to the media in tube #1. The mixture should total 100 µL. Mix well by pipetting up and down six times. This sample is the highest concentration sample (50 µg/mL/isotype) for the standard curve (top standard).

4.4 From tube #1, remove 50 µL of standard, and transfer to tube #2. Gently pipet up and down at least 6 times to completely mix the solutions.

4.5 From tube #2, transfer 50 µL volume to tube #3 and mix by pipetting. Continue transferring and mixing until you reach tube #15. Do not transfer any standard into tube #16. Tube 16 will be the media only negative control.

Note: The stock mouse IgG standard contains 200 µg /mL for each mouse IgG isotype. Following these instructions will yield a standard curve top concentration of 50 µg/mL/isotype. If a sample has IgG concentration higher than 50 µg/mL, it may NOT need an intermediate sample dilution step. Refer to **Appendix B** for more information. Additional mouse IgG standard may be obtained from iQue® (Cat. No. 91171, Cat. No. 91172, Cat. No. 91173). Please note: iQue Forecyt® software has capability to apply the standard curves from one plate with standards to any plates without standards in data analysis.

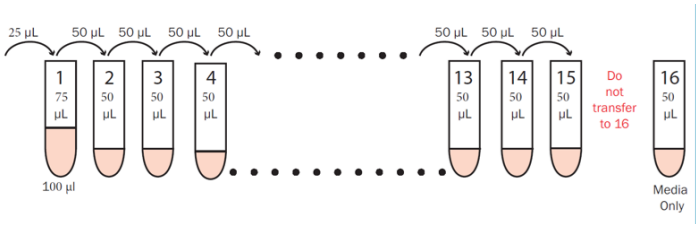


Figure 3: For 384-well Plate: 16-point Serial Dilution of IgG Standard.

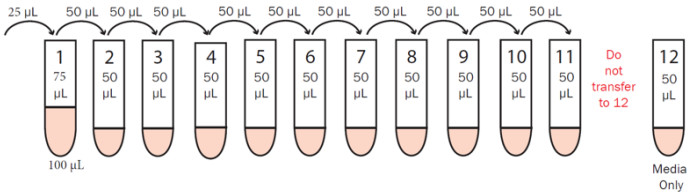


Figure 4: For 96-well Plate: 12-point Serial Dilution of IgG Standard

DO NOT add the Mouse IgG standards into the plate yet. Close the tubes containing the Mouse IgG standard to prevent evaporation; they will be transferred to the assay wells later.

Plate Design for IgG Standards

Below is an example of how to fill wells with the Mouse IgG Standards.

This design is already included in the template provided in the kit. iQue® recommends using 1–2 rows of standards and arranging the standard wells from top to bottom and from low concentration to high concentration in 384-well plate; and, from left to right and from low concentration to high concentration in a 96-well plate. In order to achieve this configuration, choose Edit Standard Set under Standards in the Design tab and choose Reverse Series.

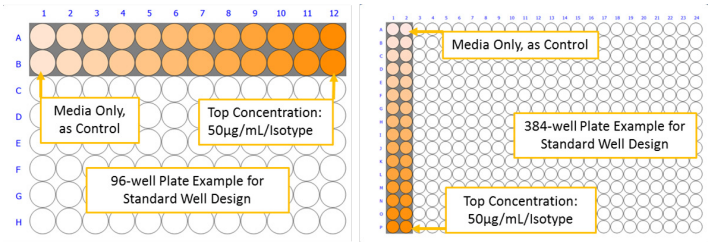


Figure 5: Arrange the standard wells from left to right (from low concentration to high concentration) in 96-well plate and from top to bottom (from low concentration to high concentration) in 384-well plate.

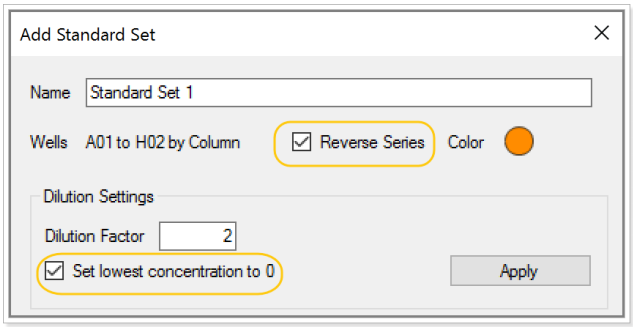


Figure 6: Reverse Series to achieve a left to right (from low concentration to high concentration) in 96-well plate and top to bottom and (from low concentration to high concentration) in 384-well plate. Check the Set lowest concentration to zero checkbox. Read the Assay Set Up before filling wells with the IgG standards.

Warning: When adding the standards into the wells as described in Assay Set Up, follow the standard sequence specified in Design during intial experiment set up. The wrong sequence will make the IgG quantitative number uninterpretable.

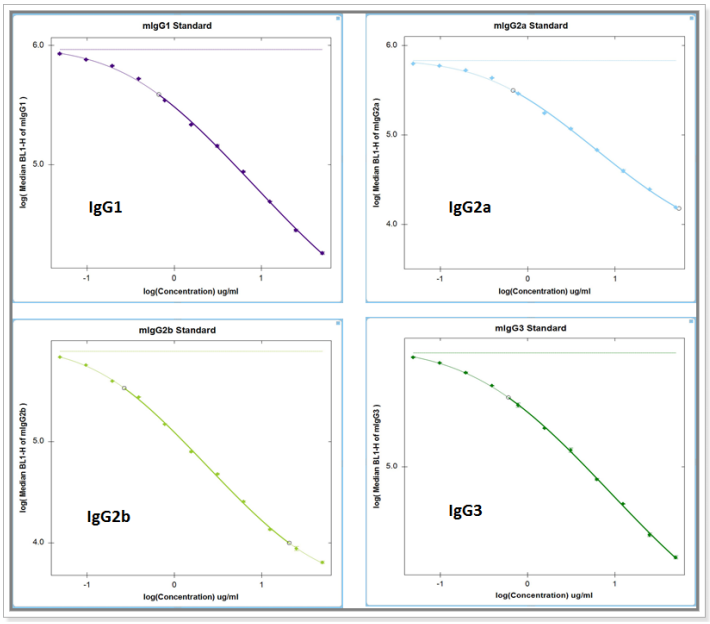


Figure 7: Representative standard curves (mouse IgG1, 2a, 2b, and 3) with 1:2 serial dilution and the top concentration at 50 µg/mL/isotype.

Assay Set Up

Total Protocol Time: 60 minutes
Total Hands-On Time: Approximately 15 minutes

This assay uses a no-wash workflow and provides results in terms of IgG concentration (e.g., µg/mL). Use the serially diluted Mouse IgG Standards to generate the 4 standard curves that are used to measure the concentration for each of the mouse IgG isotypes.

The Capture Bead vial in the kit has a mixture of 4 types of capture beads. Each capture bead type has a specific affinity for a single mouse IgG isotype. The FITC detection signal on the capture beads has an inverse relationship with the mouse IgG concentration. Total mouse IgG concentration will be calculated in each well by iQue Forecyt® by adding up the 4 mouse IgG isotype concentrations. Cell counts and cell viability will be also analyzed for each well, if the sample includes cells.

This assay protocol is designed to screen and measure a mouse hybridoma culture sample with an IgG concentration between 1 µg/mL and 50 µg/ mL. If the sample IgG range is between 1 µg/mL and 200 µg/mL, refer to **Appendix B (FAQ, Q4)** for more information on how to run a no- wash, no sample dilution assay. If the samples are from a primary mouse B cell culture with an IgG concentration between 0.1 µg/mL and 2 µg/mL, refer to **Appendix A** for high sensitivity assay protocol.

Note: 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. Add Detection Reagent Mixture (Mouse FITC-IgG and iQue® Cell Membrane Integrity (R/Red) Dye), 5 µL/well.
2. Add IgG Sample/IgG Standard, 20 µL/well; Quick spin. Mix.
3. Add prepared Capture Beads, 5 µL/well; Quick spin. Mix. Leave covered and protected from light for 60 minutes.

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

To the bottom of each well, add 5 µL of the Detection Reagent mixture (mouse FITC-IgG and iQue® Cell Membrane Integrity (R/Red) Dye) from the Detection Reagent reservoir.

2.0 Add Mouse IgG Sample | IgG Standard

2.1 Add 20 µL of mouse IgG sample (either IgG supernatant, or IgG supernatant | cell mixture) to each well designated as a sample.

The 20 µL volume is appropriate for mouse hybridoma samples with IgG concentration between 1–50 µg/mL. For samples containing between 1–200 µg/mL of IgG, refer to **Appendix B (FAQ, Q4)** for more information about designing a no-wash, no-sample-dilution measurement.

2.2 Transfer 20 µL Mouse IgG Standard prepared earlier to each well designated for IgG Standards.

2.3 After adding mouse IgG samples and standards to the plate, do a quick spin of the plate (500 x g, 5 seconds) to ensure that all solutions are at the well bottom and not attached to the well sides.

2.4 Mix the plate using the plate shaker on the iQue® platform for 20 seconds at 2,000 RPM. This ensures thorough mixing.

3.0 Add the Prepared Capture Beads Mixture

3.1 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.

3.2 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.

3.3 Mix the plate to ensure thorough mixing. Use the shaker on the iQue® platform and mix for 20 seconds at 2,000 RPM.

4.0 Incubate at room temperature for 60 minutes with lid on the plate, protected from light

After incubation, the plate is ready for sample acquisition on the iQue® platform. Make sure there is a iQue® QSol buffer cartridge in the first rinse station to prime the tubing.

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

Data Acquisition and Analysis

1.0 Launch iQue Forecyt®.

2.0 To import the template for this assay on the USB drive in the kit, insert the USB drive in your computer.

The following screenshot shows how to import the template.

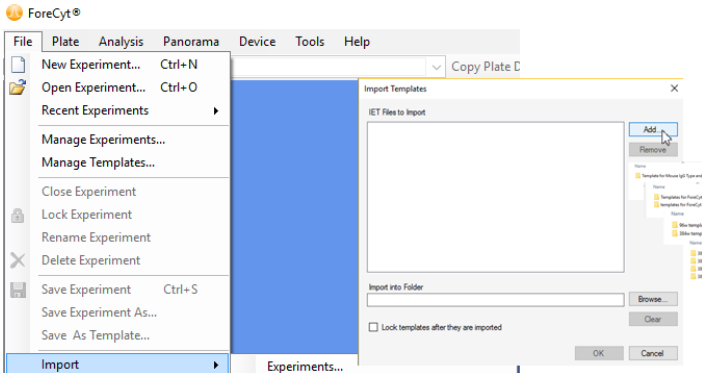


Figure 8: Import the Template

3.0 Find and select the corresponding assay template for your iQue® platform, software version and plate type (96-well or 384-well) to run this assay.

4.0 Create a New Experiment using the template.

The following screenshot shows the steps.

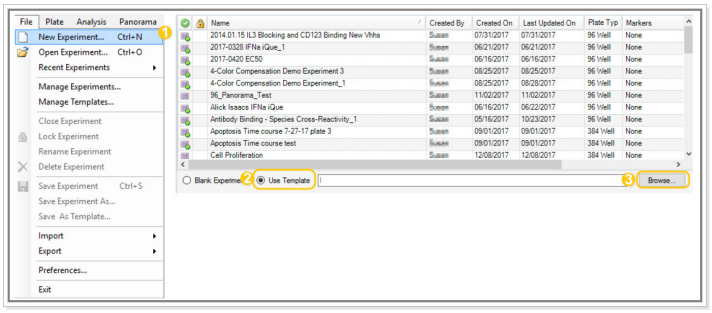


Figure 9: Create a New Experiment

5.0 The templated acquisition settings (found in the USB drive provided in the kit) are found in the bulleted list below.

- 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. The order of acquisition and how the probe moves from well to well can be specified by column or a zig-zag pattern.
- Sip Time: 2 seconds. 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® PLUS). 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. This approximate 1.5 uL volume may not be precise. For absolute event counting, please refer to **Appendix B (FAQ, Q11)**.
- 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. If you're using a 96-well plate, specify a shake speed of 2000 RPM. Inter-well Shake inserts a rinse and shake after a specified number of wells to keep sample particles in suspension. Adjust the frequency of Inter-well Shake if you find 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® PLUS. 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.

6.0 Once you've completed any Protocol adjustments, click Run on the Controller to acquire data.

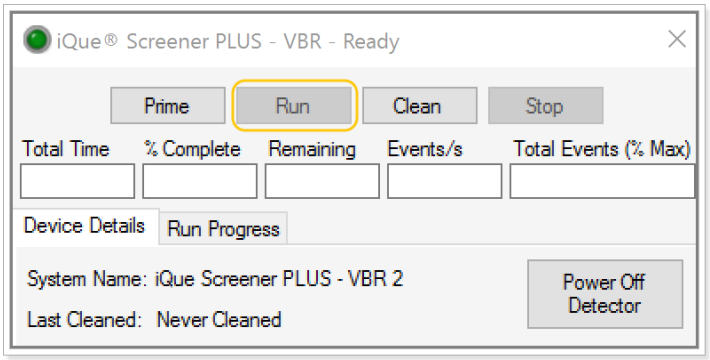


Figure 9: Click Run on the Controller

7.0 During the plate read, data will automatically begin populating into the pre-defined analysis template.

8.0 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.

8.1 **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.

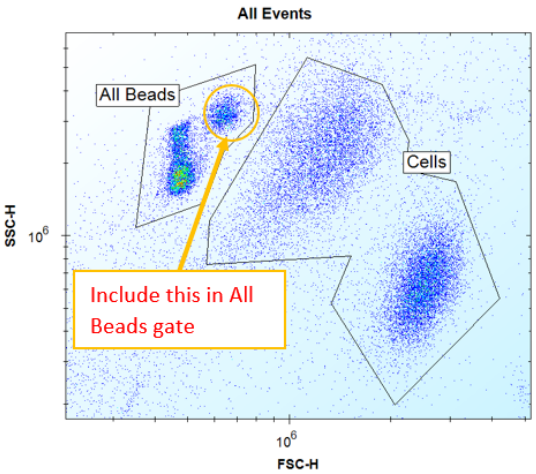


Figure 10: Gate Beads and Cells.

8.2 **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.

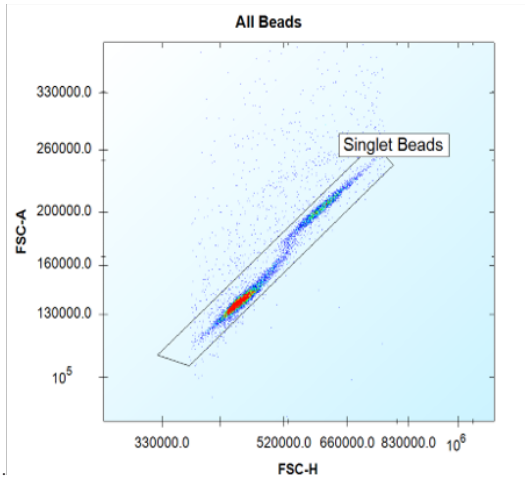


Figure 11: Singlet bead gate.

8.3 **Identify 4 Different Isotype-specific Capture Beads.** All 4 mouse IgG isotype-specific capture beads from singlet beads must be gated as shown below. For iQue® PLUS, create a RL1-H vs FSC-H plot from singlet beads, and gate 4 isotype-specific populations. As with the other pre-gated plots, make any necessary adjustments.

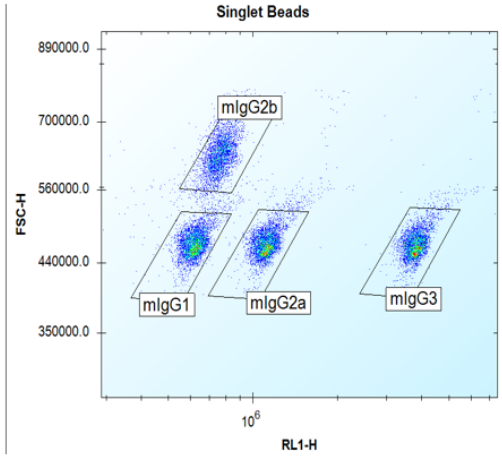


Figure 11: Gate 4 Isotype-specific Capture Beads on iQue® PLUS

For iQue®, the gating strategy is slightly different as shown below. Create a FSC-A vs SSC-A plot with the singlet beads population. Gate mouse IgG2b capture beads first (the population on the right), and then gate other 3 beads. Next, create FL4-A vs. FL3-A plot from "Other 3 beads" population, and gate mouse IgG1, IgG2a, IgG3 population from the left to the right.

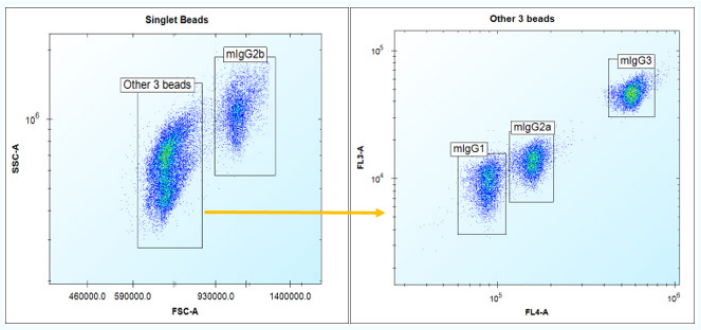


Figure 12: Gate 4 Isotype-specific Capture Beads on iQue®

8.4 **Identify Viable Cells.** Histograms show the measurement of cell viability in the RL1-H channel (iQue® PLUS) or the FL4-H channel (iQue® standard). This is a function of the membrane integrity dye binding to damaged cells' DNA. In the figure below, positive cells (those not encompassed in the Live Cell gate) represent the non-viable population. Adjust the gate (if needed) to encompass only the left-most peak. This represents the viable cells that excluded the dye. This gate separates the percentage of viable cells in each well.

Note: The iQue® Cell Membrane Integrity (R/Red) Dye used in the assay identifies both necrotic cells (highly stained population furthest to the right) and apoptotic cells (intermediate stained population between viable and necrotic cells).

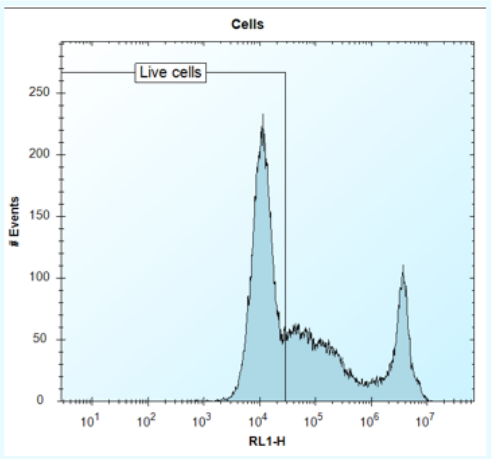


Figure 13: Viable cells on RL1-H (iQue® Plus) gated in iQue Forecyt®

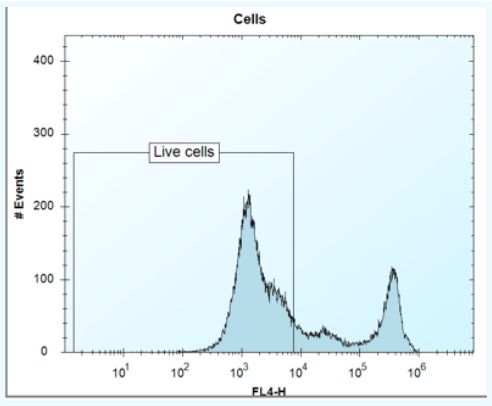
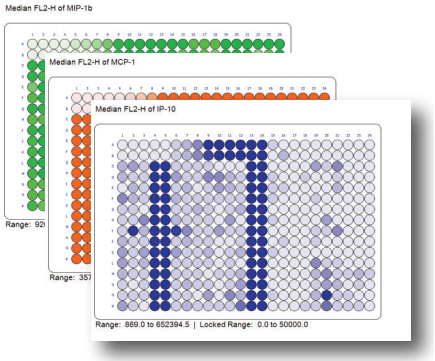


Figure 14: Viable cells FL4-H (iQue®) gated in iQue Forecyt®

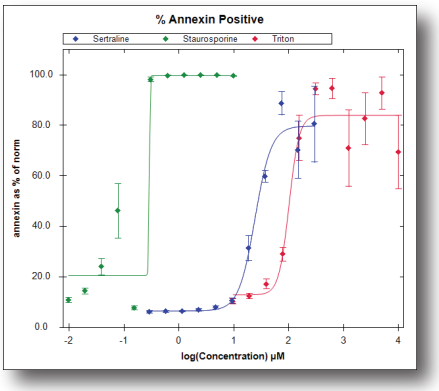
Visualization of Screening Results

1. After all the gates have been verified and adjusted as necessary for the plate-level data set, all additional analyses including heat maps, etc. will autopopulate for the specified endpoints.
2. As desired, additional data analyses and visualizations can be performed, such as dose response graphs, heatmaps, and results tables (examples below). For detailed information on additional analyses and visualizations that can be performed on this data, as well as available iQue Forecyt® software features and instructional tutorials, please visit www.intellicyt.com/resources.

Heat Maps

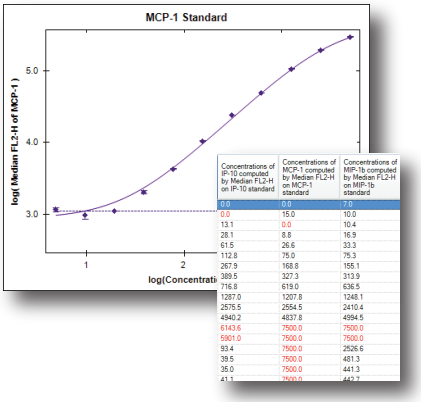


Dose Response Curves

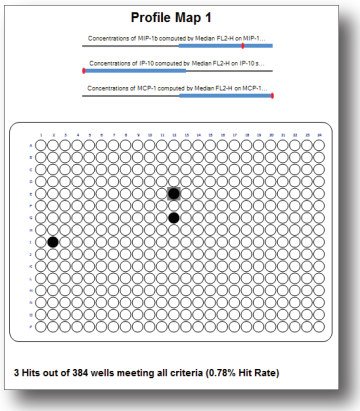


PDF Data Reports

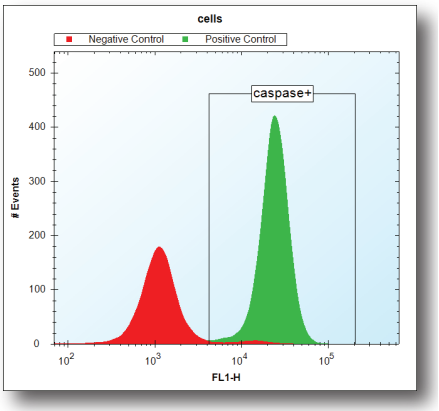
Standard Curves



Profile Maps



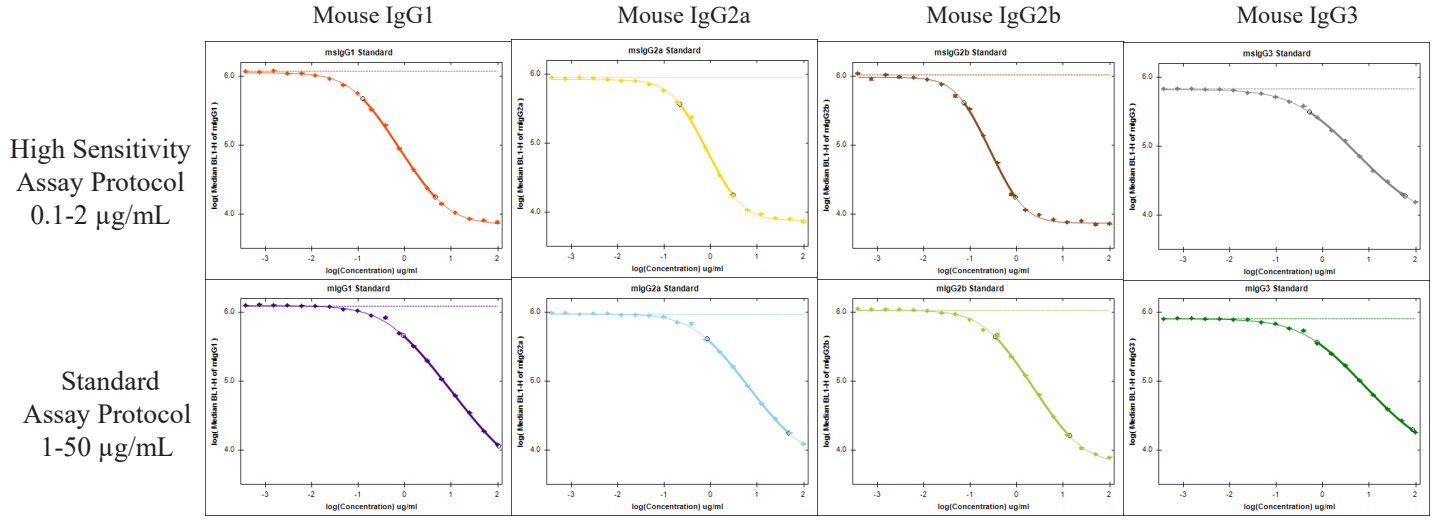
Overlay Plots



Appendix A: High Sensitivity Assay Protocol for Mouse Primary B-cell Samples

Normal hybridoma cultures contain IgG concentrations ranging from 1 to 50 µg/mL. In contrast, mouse primary B cell cultures, the IgG concentration ranges from 0.1 to 2 µg/mL. The following protocol increases the assay sensitivity allowing accurate quantification of mouse IgG in B-cell cultures:

1. Add 5 µL prepared capture beads to each well.
2. Add 20 µL prepared sample or standards to each well. Quick spin (500 x g, 5 seconds). Briefly mix the plate (2,000 RPM, 20 seconds).
3. Incubate at room temperature for 120 minutes without light.
4. Add 5 µL prepared 2 detection reagent mixture to each well. Quick spin (500 x g, 5 seconds). Briefly mix the plate (2,000 RPM, 20 seconds).
5. Incubate at room temperature for 60 minutes without light. Now the plate is ready for sampling on iQue® platform.



Appendix B: FAQ

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

Answer: When there is a straight line instead of a standard curve at saturation level, the competition assay setup is wrong. You must mix the mouse FITC-IgG with mouse IgG standard first. Add the capture beads at the end. You must strictly follow the competition setup protocol to run this assay. The assay standard curve should look like a reverse S shape, not a normal S shape. When IgG concentration goes higher, the bead detection fluorescence goes lower. If your standard curve is a normal S curve, not a reverse S curve, it is very likely your standard design is opposite. Please go to iQue Forecyt® design, click "standard" tab, and then click "Edit Standard Set", and finally check or uncheck the box "Reverse Series". If you need to change standard design in multiple plates, you may use "Copy Plate Design" function and make sure to check the box "standards" in the pop-up window.

Q2: In a multi-plate screening campaign, is there any fluorescence signal shifting on capture beads?

Answer: This assay is a no wash competition assay. After assay reaction, the fluorescence signal on the beads is stable for 2–3 hours. After 3 hours, there may be a slight increase of fluorescence signal compared with the signal at Time 0. If you have standards assigned in each plate, there will be no issue even after 6 hours because the IgG quantitation will be based on the standard curves created from the same plate and this will not change the IgG quantitation for unknown samples. However, if you only have one standard plate and do not assign standard wells in each screening plate, you may re-sample the standard plate after every 2 hours of acquisition, and then apply the standard curves generated at each specific time point to the subsequent screening plates within 2 hours acquisition time in iQue Forecyt® software.

Q3: Does this assay work with IgG quantification for other species such as rat?

Answer: No. This assay will only measure IgG from mouse species; no other species will work with this assay.

Q4: Do I need to dilute my sample if my sample IgG range is between 1–200 µg/mL?

Answer: This is a no-wash no-dilution assay for measuring mouse IgG from hybridoma samples with most common range between 1-50 µg/mL. However, if you expect your sample IgG might sometimes be within 1-200 µg/mL range, there are 2 options to handle this issue in order to maintain the no-wash, no-sample-dilution workflow:

Option 1: You may use 5 µL volume for each sample instead of 20 µL volume at the as- say step when you add the sample into the well. Then, add 15 µL fresh complete culture media to each sample well. In this add-mix workflow, you dilute your sample 4 fold but avoid the tedious intermediate dilution-and-then-transfer step. For the standard wells, you still use 20 µL volume. After IgG quantitation with standard curve, the output IgG quantity will be 4 times lower than its actual quantity, and you may use iQue Forecyt® software advanced metrics function to multiply the calculated sample IgG quantity by 4 to get actual sample IgG quantity.

Option 2: The recommended top concentration for the standard is 50 µg/mL/isotype in the protocol. However, the kit provides the standard stock at 200 µg/mL/isotype. You may use 200 µg/mL for the standard as the top concentration. The volume of mouse IgG is limited, so be conservative when preparing the dilution series for the standard curves. It is very important to use just 5 µL volume for both unknown sample and standard. In this way, the total reaction volume is 15 µL: 5µL detection reagent + 5 µL sample or standard + 5 µL capture beads. Test to make sure your plate type can accommodate 15 µL sample acquisition. Under most circumstances there is no problem in sample acquisition with a 15 µL volume for 384 well plate. However, for 96 well plate, you may need to confirm that the geometry of your plate can handle the 15µL volume in the sample acquisition on the iQue® platform. If there is a problem, you can add an additional 15 µL sample reaction buffer with BSA to each well after the assay reaction is finished, and just before sample acquisition on the iQue® platform. You may double your sip time per well if necessary in the Protocol tab of iQue Forecyt®.

Q5: 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 at all, as long as you have acquired a hundred or more total beads per well. The bead number variation does NOT change the median fluorescence intensity (MFI) of the capture beads. The IgG quantification is based on bead MFI. You can avoid bead number variation by agitating the diluted beads in reservoir periodically during the manual transferring of prepared beads to assay plate. You may also increase the sip time in the Protocol tab of iQue Forecyt® before you sample the whole plate.

Q6: I have difficulty in precisely pipetting 5 µL volume in the assay plate. How should I better handle the transferring of 5 µL volume from reservoir to assay plate?

Answer: An automated liquid handler can 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 (**Appendix E**). 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 inner wall of the well prevents the 5 µL liquid droplet from hanging on the pipette tip in- stead of releasing into the assay well. A quick spin will force the solutions into the bottom of the well.

Q7: My culture plate is a 96-well format. Can the 384-well assay kit be adapted to 96-well format?

Answer: Yes. A single 384-well assay kit can be used for 4 assay plates in a 96-well format or you may directly use 1 x 96-well kit to run 96-well plate assay. 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 assay kit provides assay templates for both 384-well format and 96-well format on the USB drive included in the kit package.

Q8: Should I clean my instrument before a screening campaign?

Answer: Routinely cleaning and maintaining the iQue® platform is very important for accurate results of your assay. Follow the hardware manual cleaning directions, regardless of the samples you run through the system. Make sure to use an iQue® QSol cartridge on the buffer station (first 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 system clogging, change the sample tubing and fluidic link (if you are using iQue® PLUS). Do a long clean and an unclog by following software instruction in the iQue Forecyt® Controller. Normally the clogging will go away. If not, repeat the long clean and unclogging processes.

Q9: Can I multiplex this assay with additional cell health endpoints or with antigen binding measurements?

Answer: Yes, it is possible. You may multiplex this assay with apoptosis measurement by using one or more dyes from iQue® Human 4-Plex Apoptosis Kit. You may also multiplex this assay with antigen binding measurement by using a fluorescent antigen or by using an unlabeled antigen plus a fluorescent antibody against your antigen. However, you may need to do extensive assay optimization of the workflow to confirm performance in your biology.

Q10: Can I fix my samples in the plate with PFA?

Answer: Yes, it is possible. You may add 10 µL 4% PFA to each well after the assay reaction and mix the plate well by shaking at 2,000 rpm for 20 seconds. After 10 minutes incubation, you may sample the plate on the iQue® platform. The fixation may extend the detection signal on the capture beads. You should validate this against your biology and your plate type materials. Some plate type materials may cross-link with biology samples with fixatives. The cell viability information will not be precise since the fixation may break the cell membrane and allow the membrane dye to stain the nuclei of healthy cells.

Q11: Can I do absolute calculation of cell density in my sample?

Answer: It is possible to improve precision in cell density calculation. We recommend using SPHERO™AccuCount Particles (Spherotech®, Cat. No. ACBP-50-10) to do the volumetric measurement on the iQue® platform. This bead has the absolute count per volume unit.

Please follow the Spherotech® protocol to mix the beads well and transfer the beads to a testing plate. We recommend running the exact sample protocol and the same plate type and the same volume in the well as used in this Mouse IgG Type and Titer assay. You may only need to run 2-3 wells at the beginning of your assay day to measure the sip volume on your iQue® platform. Only use this volume measurement on the same day of the experiment in which you wish to calculate the cell density. Please adjust your final calculation by considering the sip time (in the iQue Forecyt® Protocol) and the dilution factor of your sample in the final assay reaction volume. In order to avoid cell count variance as a result of possible cell re-attachment to the well bottom | wall, we recommend using the Greiner® cell-repellent plate (e.g., Cat. No. 651970, Cat. No. 781970) or Corning® ultra-low binding plate (e.g., Cat. No. 7007, Cat. No. 4516) for a more precise cell count.

Note: The cell viability analysis based on fluorescence dye is uses a different method than traditional Trypan Blue-based viable cell measurement. The iQue® Cell Membrane Integrity (R/Red) Dye used in this assay not only stains the necrotic cells but also the apoptotic cells. The cell viability number may be lower in this assay than a Trypan Blue-based assay.

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

The following plate types and aspiration settings have been extensively tested with the iQue Qbeads® Plexscreen and iQue Qbeads® Devscreen 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 you have a different plate washer brand or model, it is possible to approximate the aspiration settings on a different system.

It is highly recommended that wash protocols utilize the aid of 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 E: 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®).

Abbreviated List of Consumables for iQue® Platform

iQue® | iQue® PLUS and HTFC® Probes Platforms

Part	Description
90659	iQue® Probe & Tubing Assy for Gen 2 iQue® & iQue® HD – 5 Pk
91088	iQue® PLUS Probe & Tubing Assy for iQue® PLUS – 5 Pk
91093	iQue® PLUS FluidLink tubing connector – 5 Pk

iQue® | iQue® PLUS and HTFC® Solutions

Part	Description
90077	iQue® Flush Concentrate Solution for iQue® | HTFC® –5 PK (makes 1
90078	iQue® Sheath Additive Concentrate Soln for Sheath Fluid for iQue® | HTFC® | iQue®
90079	Cleaning Concentrate Solution (makes 1 Liter)
90082	Extended Flow Cell Cleaning Solution for iQue® | HTFC®
90083	iQue® QSol Buffer Cartridge – Fluidic Station (Single) for iQue®| PLUS | HD
90286	iQue® Fluidic Station Buffer Cartridge – 10 Pk
90287	iQue® QSol Buffer Cartridge – Fluidic Station (10 Pk) for iQue® | PLUS | HD
90288	iQue® Fluidic Station Flush | Cleaner Cartridge – 10 Pk
90289	iQue® Fluidic Station Water Cartridge – 10 Pk
91089	PLUS ONE Detector Maintenance Solution only for iQue® PLUS (orange)
91090	PLUS TWO Detector Maintenance Solution only for iQue® PLUS (purple)
91304	iQue® QSol Buffer Concentrate Solution (100x; makes 500 mL) - Use in Sampling Area Fluid Station for iQue® | PLUS | HD | HTFC® - Use directly in assay media | sample buffer for iQue® | HD | HTFC®

iQue® | iQue® PLUS and HTFC® Marker Beads

Part	Description
90040	FL1 In-Well Marker Beads for iQue® | HTFC® - 10 X 384 well plates
90041	FL2 In-Well Marker Beads for iQue® | HTFC® - 10 X 384 well plates
90042	FL3 In-Well Marker Beads for iQue® | HTFC®- 10 X 384 well plates
90043	FL4 In-Well Marker Beads for iQue® | HTFC®- 10 X 384-well plates
90044	In-Well Marker Beads for iQue® | HTFC® Starter Kit (4 colors)
90635	FL1 Between-Well Marker Cartridge for iQue® (iQue Forecyt® 4.0 or later req'd)
90636	FL2 Between-Well Marker Cartridge for iQue® (iQue Forecyt® 4.0 or later req'd)
90637	FL3 Between-Well Marker Cartridge for iQue® (iQue Forecyt® 4.0 or later req'd)
90638	FL4 Between-Well Marker Cartridge for iQue® (iQue Forecyt® 4.0 or later req'd)

iQue®/iQue® PLUS and HTFC® Maintenance

Part	Description
90075	iQue® | HTFC® Fluidics Maintenance Kit
90295	6 peak Validation beads (for Red Laser and FL4 Detector)
90296	8 peak Validation Beads (for Blue Laser and FL1,FL2,FL3 Detectors)
91091	PLUS Validation Beads (all channels) for iQue® PLUS
91094	iQue® PLUS Maintenance Kit
91095	iQue® PLUS Maintenance Kit - 10 pack

**Refer to www.sartorius.com\ique for complete list. Contact your local area sales representive for part number and pricing information

Sales and Service Contacts

For further contacts, visit www.sartorius.com

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