SARTORIUS

Product Guide

iQue Qbeads® Plexscreen Secreted Protein Assay Kit

Human, Mouse, and Rat Analytes

Product Information

Notice to Purchaser

The iQue Qbeads® Plexscreen Kits are a member of the iQue® product line that has been extensively tested for live cell analysis applications. iQue® screening kits are validated as complete screening assays and are optimal 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.

iQue Qbeads® are available in two general classifications; the iQue Qbeads® Plexscreen class are ready to use kits complete with buffers, detection reagents and standard analytes. The iQue Qbeads® Devscreen class including streptavidin coated beads (SAv beads for conjugation with biotinylated proteins) and SH derivatized beads (for conjugation with any protein) are building block reagents that enable an end user to build custom bead assays that can be analyzed on an iQue® platform.

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.

Kit Contents

Product Name	Format
Lyophilized Standard	1 vial per plex
Capture Beads	1 vial per plex (50x)
Detection Reagent	1 bottle, (1x ready to use)
Human Capture Bead Buffer	1 bottle
Human Assay Buffer	1 bottle
Human Capture Bead Buffer for Serum Samples	1 bottle
Human Wash Buffer	1 bottle
USB Flash Drive containing: 1. iQue Forecyt® Data Acquisition and Analysis Template 2. iQue Qbeads® Specification Sheets	

Table 1: iQue Qbeads® Human Plexscreen Kits (Cat. No. #90601-90730)

Product Name	Format
Lyophilized Standard	1 vial per plex
Capture Beads	1 vial per plex (50x)
Detection Reagent	1 bottle, (1x ready to use)
Mouse and Rat Capture Bead Buffer	1 bottle
Mouse and Rat Assay Buffer	1 bottle
Mouse and Rat Wash Buffer	1 bottle
USB Flash Drive containing: 1. iQue Forecyt® Data Acquisition and Analysis Template 2. iQue Qbeads® Specification Sheets	

Table 2: iQue Qbeads® Mouse & Rat Plexscreen Kits (Cat. No. 90824-90891)

Product Name	Cat. No. 1 x 384 wells	Cat. No. 5 x 384 wells	Storage
iQue Qbeads® Human Plexscreen Kit 1 plex	90601	90701	2–8 °C
iQue Qbeads® Human Plexscreen Kit 2 plex	90602	90702	2–8 °C
iQue Qbeads® Human Plexscreen Kit 3 plex	90603	90703	2–8 °C
iQue Qbeads® Human Plexscreen Kit 4 plex	90604	90704	2–8 °C
iQue Qbeads® Human Plexscreen Kit 5 plex	90605	90705	2–8 °C
iQue Qbeads® Human Plexscreen Kit 6 plex	90606	90706	2–8 °C
iQue Qbeads® Human Plexscreen Kit 7 plex	90607	90707	2–8 °C
iQue Qbeads® Human Plexscreen Kit 8 plex	90608	90708	2–8 °C
iQue Qbeads® Human Plexscreen Kit 9 plex	90609	90709	2–8 °C
iQue Qbeads® Human Plexscreen Kit 10 plex	90610	90710	2–8 °C
iQue Qbeads® Human Plexscreen Kit 11 plex	90611	90711	2–8 °C
iQue Qbeads® Human Plexscreen Kit 12 plex	90612	90712	2–8 °C
iQue Qbeads® Human Plexscreen Kit 13 plex	90613	90713	2–8 °C
iQue Qbeads® Human Plexscreen Kit 14 plex	90614	90714	2–8 °C
iQue Qbeads® Human Plexscreen Kit 15 plex	90615	90715	2–8 °C
iQue Qbeads® Human Plexscreen Kit 16 plex	90616	90716	2–8 °C
iQue Qbeads® Human Plexscreen Kit 17 plex	90617	90717	2–8 °C
iQue Qbeads® Human Plexscreen Kit 18 plex	90618	90718	2–8 °C
iQue Qbeads® Human Plexscreen Kit 19 plex	90619	90719	2–8 °C
iQue Qbeads® Human Plexscreen Kit 20 plex	90620	90720	2–8 °C
iQue Qbeads® Human Plexscreen Kit 21 plex	90621	90721	2–8 °C
iQue Qbeads® Human Plexscreen Kit 22 plex	90622	90722	2–8 °C
iQue Qbeads® Human Plexscreen Kit 23 plex	90623	90723	2–8 °C
iQue Qbeads® Human Plexscreen Kit 24 plex	90624	90724	2–8 °C
iQue Qbeads® Human Plexscreen Kit 25 plex	90625	90725	2–8 °C
iQue Qbeads® Human Plexscreen Kit 26 plex	90626	90726	2–8 °C
iQue Qbeads® Human Plexscreen Kit 27 plex	90627	90727	2–8 °C
iQue Qbeads® Human Plexscreen Kit 28 plex	90628	90728	2–8 °C
iQue Qbeads® Human Plexscreen Kit 29 plex	90629	90729	2–8 °C
iQue Qbeads® Human Plexscreen Kit 30 plex	90630	90730	2–8 °C

Table 3: List of Human Plexscreen Catalog Numbers

Product Name	Cat. No. 1 x 384 wells	Cat. No. 5 x 384 wells	Storage
iQue Qbeads® Mouse Plexscreen Kit 1 plex	90824	90852	2–8 °C
iQue Qbeads® Mouse Plexscreen Kit 2 plex	90825	90853	2–8 °C
iQue Qbeads® Mouse Plexscreen Kit 3 plex	90826	90854	2–8 °C
iQue Qbeads® Mouse Plexscreen Kit 4 plex	90827	90855	2–8 °C
iQue Qbeads® Mouse Plexscreen Kit 5 plex	90828	90856	2–8 °C
iQue Qbeads® Mouse Plexscreen Kit 6 plex	90829	90857	2–8 °C
iQue Qbeads® Mouse Plexscreen Kit 7 plex	90830	90858	2–8 °C
iQue Qbeads® Mouse Plexscreen Kit 8 plex	90831	90859	2–8 °C
iQue Qbeads® Mouse Plexscreen Kit 9 plex	90832	90860	2–8 °C
iQue Qbeads® Mouse Plexscreen Kit 10 plex	90833	90861	2–8 °C
iQue Qbeads® Mouse Plexscreen Kit 11 plex	90834	90862	2–8 °C
iQue Qbeads® Mouse Plexscreen Kit 12 plex	90835	90863	2–8 °C
iQue Qbeads® Mouse Plexscreen Kit 13 plex	90836	90864	2–8 °C
iQue Qbeads® Mouse Plexscreen Kit 14 plex	90837	90865	2–8 °C
iQue Qbeads® Mouse Plexscreen Kit 15 plex	90838	90866	2–8 °C
iQue Qbeads® Mouse Plexscreen Kit 16 plex	90839	90867	2–8 °C
iQue Qbeads® Mouse Plexscreen Kit 17 plex	90840	90868	2–8 °C
iQue Qbeads® Mouse Plexscreen Kit 18 plex	90841	90869	2–8 °C
iQue Qbeads® Mouse Plexscreen Kit 19 plex	90842	90870	2–8 °C
iQue Qbeads® Mouse Plexscreen Kit 20 plex	90843	90871	2–8 °C
iQue Qbeads® Mouse Plexscreen Kit 21 plex	90844	90872	2–8 °C
iQue Qbeads® Mouse Plexscreen Kit 22 plex	90845	90873	2–8 °C
iQue Qbeads® Mouse Plexscreen Kit 23 plex	90846	90874	2–8 °C
iQue Qbeads® Mouse Plexscreen Kit 24 plex	90847	90875	2–8 °C
iQue Qbeads® Mouse Plexscreen Kit 25 plex	90848	90876	2–8 °C
iQue Qbeads® Mouse Plexscreen Kit 26 plex	90849	90877	2–8 °C
iQue Qbeads® Mouse Plexscreen Kit 27 plex	90850	90878	2–8 °C
iQue Qbeads® Mouse Plexscreen Kit 28 plex	90851	90879	2–8 °C

Table 4: List of Mouse Plexscreen Catalog Numbers

Product Name	Cat. No. 1 x 384 wells	Cat. No. 5 x 384 wells	Storage
iQue Qbeads® Rat Plexscreen Kit 1 plex	90880	90886	2–8 °C
iQue Qbeads® Rat Plexscreen Kit 2 plex	90881	90887	2–8 °C
iQue Qbeads® Rat Plexscreen Kit 3 plex	90882	90888	2–8 °C
iQue Qbeads® Rat Plexscreen Kit 4 plex	90883	90889	2–8 °C
iQue Qbeads® Rat Plexscreen Kit 5 plex	90884	90890	2–8 °C
iQue Qbeads® Rat Plexscreen Kit 6 plex	90885	90891	2–8 °C

Table 5: List of Rat Plexscreen Catalog Numbers

Detection Channels

Detector (nm)	Spectrum	Blue Laser (488 nm)		Red Laser (640 nm)	
533/30		(B/ Green)			
585/40		(B/ Yellow)	Detection Channel		
670 LP		(B/Red)			
675/25				(R/Red)	Classification Channel: X-axis
780/60				(B/Red) (SDS)	Classification Channel: Y-axis

Table 3. iQue® Standard Detector Channels

Note: The use of iQue Qbeads® Devscreen SH beads on the iQue® requires the use of the Selectable Detector Set (SDS) feature. Before beginning, ensure that your system is licensed for SDS and that the alternate emission filter (780/60 nm) is installed in the B/Red position. Configure the SDS setting to the 2:2 Detector option by following the software wizard in iQue Forecyt®.

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)			
572/28		(V/ Yellow)	(B/ Yellow)	Detection Channel		
615/24		(V/ Orange)	(B/ Orange)			
675/30		(V/Red)	(B/Red)		(R/Red)	Classification Channel: X-axis
780/60		(V/ Crimson)	(B/ Crimson)		(R/ Crimson)	Classification Channel: Y-axis

Table 4. iQue® PLUS Detector Channels

Materials Needed but Not Provided

- iQue® platform
- iQue Forecyt®, versions 3.1 or higher

  Only iQue Forecyt® versions 4.1 or higher will support quantitation of standard curves and extrapolation of unknown samples in concentration quantities.
- 384-well assay plates (recommended: Greiner® 781280)
- Selectable Detector Set (SDS) software license
- Alternate Emission Filter 780/60 nm

  Note: The SDS software license and alternate emission filter are available as the iQue Qbeads® Enabler Bundle (Cat. No.: 90331)

Additional Materials Needed but Not Provided for "Reduced Background" Protocols:

- Centrifuge capable of spinning microplates
- Plate washer

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Background

Many proteins secreted by cells are involved in the process of cell signaling and signal transduction. Aberrant production and | or secretion of proteins have long-reaching effects for physiological processes, and dysfunctional signaling is implicated in virtually all diseases. The detection and quantification of soluble proteins in solution provides key information for researchers in therapeutic areas ranging from cancer and immunology to aging and regeneration. Characterizing the complex signaling pathways between cells is an important target across the drug discovery process, from primary screening to toxicity profiling.

iQue Qbeads® Plexscreen beads are a family of beads that are designed for ease of use and multiplexing. Analytes targeted against human, mouse, and rat are available to enhance enhance the flexibility of this platform. The iQue Qbeads® Plexscreen product family is turnkey ready-to-use, and is capable of qualitative and quantitative analysis of multiple analytes from a single sample. In addition to supporting highly customizable configurations, this panel features:

- Multiplexing with up to 30 different analytes in a single sample using the same bead classification scheme
- Seamless multiplexing with other products in iQue® reagent family, including iQue Qbeads® Devscreen bead products
- No wash assay protocols that support multiplexing with cellular endpoints, for simultaneous measurements of beads and cells

iQue Qbeads® Plexscreen assays are specifically and exclusively designed for use on iQue® platforms. These systems and the iQue Qbeads® combined are a powerful platform that enables a streamlined no-wash workflow, highly customizable assays, and assay miniaturization.

Assay Principles

iQue Qbeads® function on the same principles as a sandwich ELISA. Using an example of a single analyte (**Figure 1**), capture beads coated with capture antibodies directed against an analyte of interest are directly combined with the sample. Samples can be from cell culture supernatant or serum | plasma. Once the analyte is bound by the capture beads, a fluorescent detection antibody is added to the reaction which then binds the analyte forming a "sandwich." The fluorescence signal is now associated with the bead complex, with the intensity of fluorescence directly correlating to the quantity of bound analyte.

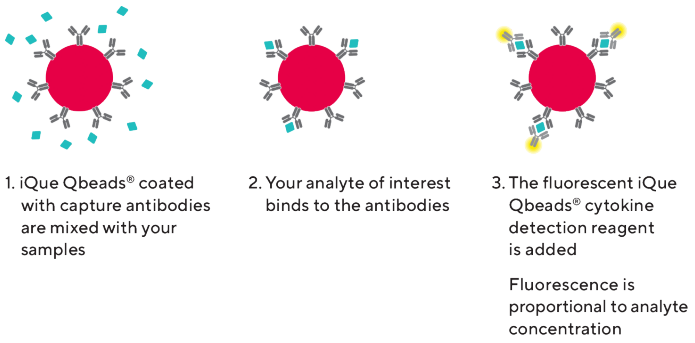


Figure 1. Schematic of a bead-based sandwich ELISA showing a single capture bead detecting a single analyte. Once fully formed, the fluorescence signal is directly associated with the bead, and the intensity of the signal is proportional to the bound analyte concentration.

Quantitative readouts from this assay can be measured as fluorescence intensity, or interpolated to a concentration (pg/mL) in solution via the use of a standard curve. Specific beads designed to capture different analytes are also fluorescently tagged with a unique signature, allowing for up to 30 beads to be combined in a single sample. The iQue® platform then discriminates the various bead sub-populations and the associated analyte during analysis.

Mix-and-Read Assay Workflow Overview

iQue Qbeads® uniquely enable a screening-friendly, no-wash protocol. If desired, greater low-end sensitivity can be achieved for some analytes by performing a single wash step to reduce background just prior to reading on the iQue® platform.

iQue Qbeads® No-Wash Screening Workflow

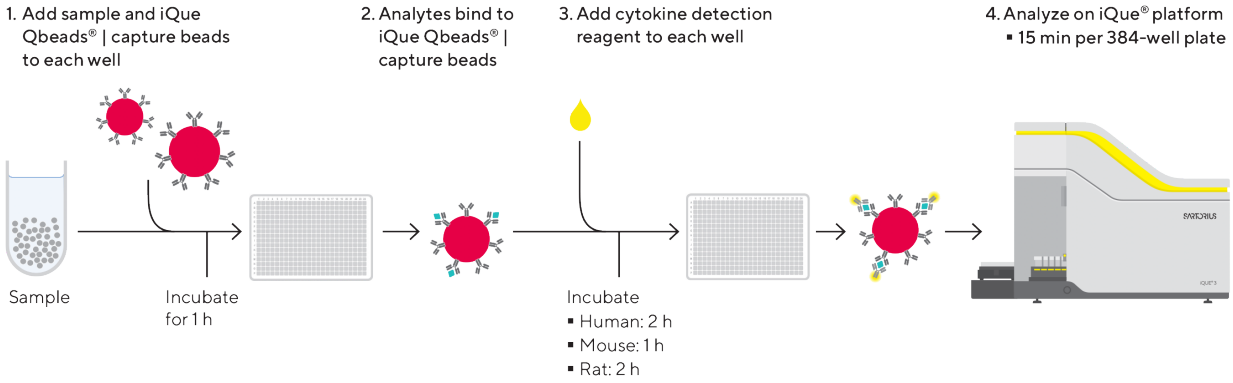


Figure 2. A schematic of the iQue Qbeads® workflow on iQue® platforms. iQue Qbeads® enable a simplified, no-wash workflow. Combine 10 µL of mixed beads and 10 µL of sample, then incubate for 1 hour. Add 10 µL of detection reagent and incubate for 2 hours (for human and rat analytes, or 1 hour for mouse analytes). Finally, directly read the plate on an iQue® platform. Plate reads typically take less than 20 minutes, regardless of the number of analytes that are being detected.

Before Beginning

If performing assay on a standard iQue® platform, enable the instrument to measure iQue Qbeads® by installing the 780/60 filter in the B/Red position, and change the SDS configuration to 2:2. Follow the SDS wizard for this procedure. iQue Qbeads® will not work without these changes. It is recommended that the changed configuration be noted on the instrument to prevent unintended use of this configuration. No instrument configuration changes are necessary if running the iQue Qbeads® on an iQue® PLUS platform.

- Briefly centrifuge all vials before use to prevent reagent loss.
- Vigorously vortex beads to ensure a homogenous bead solution and consistent concentration in assay. Beads tend to settle and aggregate over time.
- Identify the desired protocol from Protocol A, B, C, and D, briefly described below.

❒ Protocol A: Screening

Total Protocol Time: Approximately 3 hours 15 minutes (2:15 for mouse)

Total Hands-On Time: Approximately 15 minutes
This simplified protocol generates relative amounts of analytes in terms of median fluorescence intensity (MFI) which can be compared to controls. This is a no-wash protocol and represents the quickest method to measure relative quantities of protein in a sample.

❒ Protocol B: Screening, Standard Curve

Total Protocol Time: Approximately 3 hours 30 minutes (2:30 for mouse)

Total Hands-On Time: Approximately 30 minutes
This simplified protocol takes advantage of the no-wash work flow and allows for generation of results in terms of analyte concentration (e.g., pg/mL). This protocol involves preparing a serial titration of a reference protein to generate a standard curve which is used to determine analyte concentrations.

❒ Protocol C: Reduced Background

Total Protocol Time: Approximately 3 hours 30 minutes (2:30 for mouse)

Total Hands-On Time: Approximately 30 minutes

Requires: Centrifuge for microplates and plate washer
This protocol generates relative secretion of analytes in terms of median fluorescence intensity (MFI) which can be compared to controls. It maximizes analyte sensitivity by washing the bead | analyte after incubation of the beads with detection reagent. The single-wash step reduces background intensity, enabling better discrimination of low analyte concentrations.

❒ Protocol D: Reduced Background, Standard Curve

Total Protocol Time: Approximately 3 hours 45 minutes (2:45 for mouse)

Total Hands-On Time: Approximately 45 minutes

Requires: Centrifuge for microplates and plate washer

This protocol allows for generation of results in terms of analyte concentration (e.g., pg/mL). It involves preparing a serial titration of a reference protein to generate a standard curve which can be used to determine analyte concentrations . It maximizes analyte sensitivity by washing the bead | analyte after incubation of the beads with detection reagent. The single wash step reduces background intensity, enabling better discrimination of low analyte concentrations.

Protocol A: Screening

1.0 Preparing Samples

1.1 Determine the detection range for all analytes to be tested from the provided specification sheets.

1.2 Consider diluting samples that are expected to be outside of the assays' detection range.

1.3 Obtain a blank (zero analyte) control that is prepared in the same diluent or media as the actual samples.

2.0 Prepare Capture Beads

The capture beads for each analyte in the kit are supplied as 50x concentrates in individual tubes, one tube per plex. They must be combined and diluted to working concentration just before use. The instructions below are for the preparation of enough capture beads to run one 384-well plate with minimal overage.

2.1 Vigorously vortex the capture bead tubes for each analyte.

2.2 In a 15 mL conical tube, combine 90 µL of beads for each analyte.

The volumes above are specified to create enough prepared reagent for adding 10 µL per well for a full plate with minimal overage. To prepare reagent for partial plates or with more overage, refer to **Appendix C.**

2.3 Bring the final volume of the bead mixture to 4.5 mL with the appropriate capture bead buffer. For human samples utilizing serum or plasma, ensure that the capture bead buffer for serum | plasma is used. All other sample types (including mouse serum | plasma samples) should utilize the standard capture bead buffer. Note that the amount of buffer added will vary according to the number of plexes for the specific assay.

2.4 Vortex beads briefly to mix well and ensure that they form a homogenous mixture.

3.0 Perform the Assay

3.1 Capture Analytes onto Beads

3.1.1 In each well of a 384-well plate, combine 10 µL of prepared beads with 10 µL of sample or standards. Ensure that samples are at the bottom of the wells, not attached to the sides.

3.1.2 Mix the plate using a plate shaker and ensure thorough mixing. The shaker on the iQue® platform can be utilized for this step (**Appendix A** for appropriate shake speeds).

3.1.3 Incubate the plate at room temperature for 1 hour, protected from light.

3.2 Add Detection Reagent

3.2.1 Add 10 µL of detection reagent to each well.

3.2.2 Mix the plate using a plate shaker and ensure thorough mixing. The shaker on the iQue® platform can be utilized for this step (**Appendix A** for appropriate shake speeds).

3.2.3 If working with human or rat analytes, incubate the plate at room temperature for 2 hours, protected from light.

If working with mouse analytes, incubate the plate at room temperature for 1 hour, protected from light.

3.3 Acquire Data

Once the incubation is complete, directly acquire data on the iQue® platform **(Page 7)**.

Protocol B: Screening, Standard Curve

1.0 Preparing Samples

1.1 Determine the detection range for all analytes to be tested from the provided specification sheets.

1.2 Ensure that the samples are in the detectable range for all analytes, or as needed, dilute samples in assay buffer if quantitative results are desired.

1.3 Obtain a blank (zero analyte) control that is prepared in the same diluent or media as the actual samples.

2.0 Prepare Protein Standards

The protein standards for this kit are provided as individually packaged lyophilized spheres, with one vial provided per plex. The vials are sealed under nitrogen, and care should be exercised when opening vials to prevent loss of the spheres.

Before opening vials, visually inspect each vial and confirm the presence of the sphere. The lyophilized spheres are lightweight and may be attached to the side of the vial or under the rubber stopper in the cap. Gently tap the unopened vial several times to ensure that the sphere is at the bottom of the vial before opening.

2.1 Carefully combine all the lyophilized standards into a 15-mL conical tube. Take precautions to ensure that spheres do not get crushed or damaged during this transfer step, and that all spheres have been transferred by counting the total number of spheres present in the conical tube once complete. If assay sterility is not required, this step may be conducted outside of a biosafety hood to minimize the airflow disturbances as the lyophilized spheres are extremely lightweight and electrostatic.

2.2 Carefully add 2 mL of assay buffer to the vial, and ensure that all standard spheres are reconstituted. Gentle pipetting might be necessary if any lyophilized spheres are adhered to the sidewall of the conical tube. Visually inspect tube to ensure that all the spheres are dissolved.

2.3 Let stand for 15 minutes at room temperature to fully reconstitute. Gently mix the solution by inversion. Do not vortex as this will cause the solution to foam.

3.0 Prepare Capture Beads

The capture beads for each analyte in the kit are supplied as 50x concentrates in individual tubes, one tube per plex. They must be combined and diluted to working concentration just before use. The instructions below are for the preparation of enough capture beads for one 384-well plate with minimal overage.

3.1 Vigorously vortex the capture bead tubes for each analyte.

3.2 In a 15 mL conical tube, combine 90 µL of beads for each analyte.

Note: The volumes above are specified to create enough prepared reagent for adding 10 µL per well for a full plate with minimal overage. To prepare reagent for partial plates or with more overage, refer to **Appendix C.**

3.3 Bring the final volume of the bead mixture to 4.5 mL with the appropriate capture bead buffer. For human samples utilizing serum or plasma, ensure that the capture bead buffer for serum | plasma is used. All other sample types (including mouse serum | plasma samples) should utilize the standard capture bead buffer. Note that the amount of buffer added will vary according to the number of plexes for the specific assay.

3.4 Vortex beads briefly to mix well and ensure that they form a homogenous mixture.

4.0 Perform the Assay

4.1 Capture Analytes onto Beads

4.1.1 In each well of a 384-well plate, combine 10 µL of prepared beads with 10 µL of sample or standards. Ensure that samples are at the bottom of the wells, not attached to the sides.

4.1.2 Mix the plate using a plate shaker and ensure thorough mixing. The shaker on the iQue® platform can be utilized for this step (**Appendix A** for appropriate shake speeds).

4.1.3 Incubate the plate at room temperature for 1 hour, protected from light.

4.2 Add Detection Reagent

4.2.1 Add 10 µL of detection reagent to each well.

4.2.2 Mix the plate using a plate shaker and ensure thorough mixing. The shaker on the iQue® platform can be utilized for this step (**Appendix A** for appropriate shake speeds).

4.2.3 If working with human or rat analytes, incubate the plate at room temperature for 2 hours, protected from light.

If working with mouse analytes, incubate the plate at room temperature for 1 hour, protected from light.

4.3 Acquire Data

Once the incubation is complete, directly acquire data on the iQue® platform **(Page 7)**.

Protocol C: Reduced Background

1.0 Preparing Samples

1.1 Determine the detection range for all analytes to be tested from the provided specification sheets.

1.2 Consider diluting samples that are expected to be outside of the assays' detection range.

1.3 Obtain a blank (zero analyte) control that is prepared in the same diluent or media as the actual samples.

2.0 Prepare Capture Beads

The capture beads for each analyte in the kit are supplied as 50x concentrates in individual tubes, one tube per plex. They must be combined and diluted to working concentration just before use. The instructions below are for the preparation of enough capture beads for one 384-well plate with minimal overage.

2.1 Vigorously vortex the capture bead tubes for each analyte.

2.2 In a 15 mL conical tube, combine 90 µL of beads for each analyte.

Note: The volumes above are specified to create enough prepared reagent for adding 10 µL per well for a full plate with minimal overage. To prepare reagent for partial plates or with more overage, refer to **Appendix C.**

2.3 Bring the final volume of the bead mixture to 4.5 mL with the appropriate capture bead buffer. For human samples utilizing serum or plasma, ensure that the capture bead buffer for serum | plasma is used. All other sample types (including mouse serum | plasma samples) should utilize the standard capture bead buffer. Note that the amount of buffer added will vary according to the number of plexes for the specific assay.

2.4 Vortex beads briefly to mix well and ensure that they form a homogenous mixture.

3.0 Perform the Assay

3.1 Capture Analytes onto Beads

3.1.1 In each well of a 384-well plate, combine 10 µL of prepared beads with 10 µL of sample or standards. Ensure that samples are at the bottom of the wells, not attached to the sides.

3.1.2 Mix the plate using a plate shaker and ensure thorough mixing. The shaker on the iQue® platform can be utilized for this step (**Appendix A** for appropriate shake speeds).

3.1.3 Incubate the plate at room temperature for 1 hour, protected from light.

3.2 Add Detection Reagent

3.2.1 Add 10 µL of detection reagent to each well.

3.2.2 Mix the plate using a plate shaker and ensure thorough mixing. The shaker on the iQue® platform can be utilized for this step (**Appendix A** for appropriate shake speeds).

3.2.3 If working with human or rat analytes, incubate the plate at room temperature for 2 hours, protected from light.

If working with mouse analytes, incubate the plate at room temperature for 1 hour, protected from light.

3.3 Wash Beads

3.3.1 Add 50 µL of wash buffer to each well.

3.3.2 Centrifuge the plate for 5 minutes at 1100 x g.

3.3.3 Aspirate supernatants. (**Appendix B** for recommendations.)

3.3.4 Resuspend samples by adding 10 µL of wash buffer to each well.

3.3.5 Mix well to ensure complete resuspension. The shaker on the iQue® platform can be utilized for this step (**Appendix A** for appropriate shake speeds).

4.3 Acquire Data

Once the incubation is complete, directly acquire data on the iQue® platform (**Page 7**).

Protocol D: Reduced Background, Standard Curve

1.0 Preparing Samples

1.1 Determine the detection range for all analytes to be tested from the provided specification sheets.

1.2 Ensure that the samples are in the detectable range for all analytes, or as needed, dilute samples in assay buffer if quantitative results are desired.

1.3 Obtain a blank (zero analyte) control that is prepared in the same diluent or media as the actual samples.

2.0 Prepare Protein Standards

The protein standards for this kit are provided as individually packaged lyophilized spheres, with one vial provided per plex. The vials are sealed under nitrogen, and care should be exercised when opening vials to prevent loss of the spheres.

Before opening vials, visually inspect each vial and confirm the presence of the sphere. The lyophilized spheres are lightweight and may be attached to the side of the vial or under the rubber stopper in the cap. Gently tap the unopened vial several times to ensure that the sphere is at the bottom of the vial before opening.

2.1 Carefully combine all the lyophilized standards into a 15-mL conical tube. Take precautions to ensure that spheres do not get crushed or damaged during this transfer step, and that all spheres have been transferred by counting the total number of spheres present in the conical tube once complete.

If assay sterility is not required, this step may be conducted outside of a biosafety hood to minimize the airflow disturbances as the lyophilized spheres are extremely lightweight and electrostatic.

2.2 Carefully add 2 mL of assay buffer to the vial, and ensure that all standard spheres are reconstituted. Gentle pipetting might be necessary if any lyophilized spheres are adhered to the sidewall of the conical tube. Visually inspect tube to ensure that all the spheres are dissolved.

2.3 Let stand for 15 minutes at room temperature to fully reconstitute. Gently mix the solution by inversion. Do not vortex as this will cause the solution to foam.

3.0 Prepare Capture Beads

The capture beads for each analyte in the kit are supplied as 50x concentrates in individual tubes, one tube per plex. They must be combined and diluted to working concentration just before use. The instructions below are for the preparation of enough capture beads for one 384-well plate, with minimal overage.

3.1 Vigorously vortex the capture bead tubes for each analyte.

3.2 In a 15 mL conical tube, combine 90 µL of beads for each analyte.

Note: The volumes above are specified to create enough prepared reagent for adding 10 µL per well for a full plate with minimal overage. To prepare reagent for partial plates or with more overage, refer to **Appendix C.**

3.3 Bring the final volume of the bead mixture to 4.5 mL with the appropriate capture bead buffer. For human samples utilizing serum or plasma, ensure that the capture bead buffer for serum | plasma is used. All other sample types (including mouse serum | plasma samples) should utilize the standard capture bead buffer. Note that the amount of buffer added will vary according to the number of plexes for the specific assay.

3.4 Mix the beads by vortexing to ensure that they are suspended.

4.0 Perform the Assay

4.1 Capture Analytes onto Beads

4.1.1 In each well of a 384-well plate, combine 10 µL of prepared beads with 10 µL of sample or standards. Ensure that samples are at the bottom of the wells, not attached to the sides.

4.1.2 Mix the plate using a plate shaker and ensure thorough mixing. The shaker on the iQue® platform can be utilized for this step (**Appendix A** for appropriate shake speeds).

4.1.3 Incubate the plate at room temperature for 1 hour, protected from light.

4.2 Add Detection Reagent

4.2.1 Add 10 µL of detection reagent to each well.

4.2.2 Mix the plate using a plate shaker and ensure thorough mixing. The shaker on the iQue® platform can be utilized for this step (**Appendix A** for appropriate shake speeds).

4.3.3 If working with human or rat analytes, incubate the plate at room temperature for 2 hours, protected from light.

If working with mouse analytes, incubate the plate at room temperature for 1 hour, protected from light.

4.3 Wash Beads

4.3.1 Add 50 µL of wash buffer to each well.

4.3.2 Centrifuge the plate for 5 minutes at 1100 x g.

4.3.3 Aspirate supernatants (**Appendix B** for recommendations).

4.3.4 Resuspend samples by adding 10 µL of wash buffer to each well.

4.3.5. Mix well to ensure complete resuspension. The shaker on the iQue® platform can be utilized for this step (15 sec. at 2800 RPM).

4.4 Acquire Data

Once the incubation and wash are complete, directly acquire data on the iQue® platform **(Page 7**).

Data Acquisition and Analysis

1. Launch iQue Forecyt® (version 3.1 or greater).
2. Create a new experiment using the customized assay template provided on the USB flash drive in the kit. The template provided is lot specific, and unique to the specific plex size and analyte set.

   The USB drive will contain several different versions of the template, for the different iQue® platforms. Select the template and software version that is appropriate for your system. Note that the improper selection of a template will cause an error in iQue Forecyt® stating that the template is incompatible. iQue® PLUS and iQue® standard templates are not cross-compatible.

   Additionally, older versions of iQue Forecyt® will not be able to utilize templates generated for newer versions.

   The template contains a preset sampling protocol for data acquistion and provides an automated analysis and annotations for the analytes that are specific for each kit. A new experiment based on a template will populate with data after the plate read has started.

   Note: The recommended order for sampling of dose responses is from low to high concentration. Additionally, an interwell shake is recommended after the high dose in each series to prevent any potential signal carry-over to lower dose wells. See below for the recommended interwell shake parameters.

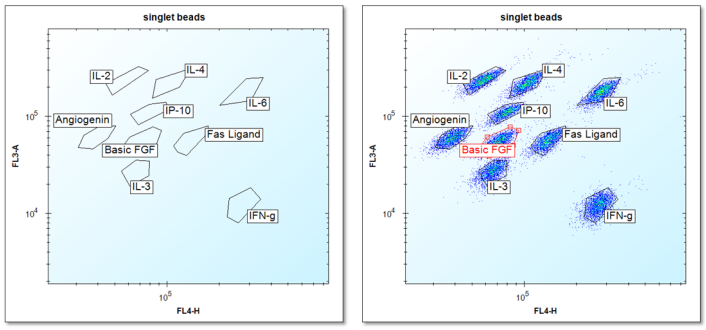


Figure 3. A template is provided with each kit that predefines an analysis for the included analytes. Shown above are gates that identify bead populations. These images will differ from a user's template based on the analytes in their kit. Before acquisition, the bead population gates will be empty (left panel). During acquisition, the gates will populate with the appropriate beads as they are read. If a gate does not completely encompass a bead population, click the gate label to shift the gate.

   For detailed instructions on how to load and | or use iQue® assay templates, refer to the iQue® Video Tutorial Series found at http://intellicyt.com/resources.

   The template contains a preset sampling protocol for data acquisition, but changes to the protocol can and should be made to optimize the sampling for the specific experimental setup. For instance, the recommended order for sampling of dose responses is from low to high concentration and the sampling direction should be adjusted accordingly.

   Additionally, an interwell shake is recommended after the high dose in each series to prevent any potential signal carry-over to lower dose wells. The shaking interval on the protocol should be adjusted for the specific number of wells in each dose series.

   Note: All iQue Qbeads® templates are custom generated for the specific plex size and analytes being tested. If the kit-specific template cannot be located, contact iQue® technical support for a replacement.

3. The default acquisition settings in bold are briefly explained below. Note that these settings will vary depending on the system used for acquisition, and are guidelines based on the assay protocol. Modifications to the protocol could require optimization of sampling parameters for best results. If necessary, potential optimizations to each setting are given.

   - Pre-Plate Prime: 60 seconds; Prior to sampling a plate, the system will prime the tubing with the S1 rinse station buffer for the specified amount of time. The time can be decreased if a faster acquisition is desired and | or tubing is already clean. Increasing the prime time is not recommended.
   - Pre-Plate Shake: 30 seconds at 3000 RPM; Prior to sampling a plate, the orbital shaker will agitate the samples at the specified speed and time. The specified shake speed may vary depending on the system the assay is to be performed on. Use the default shake speed specified with the supplied template.
   - Sampling Order: by row; The order of acquisition and how the probe moves from well to well can be specified. Can be set to "by column" or a zig-zag pattern.
   - Sip Time: 1 second; Specifies the amount of time the probe spends in each well. Sip time is proportional to the sample volume collected per well. Sip time can be increased to acquire more data points per well. The tradeoff is slower overall read-time.

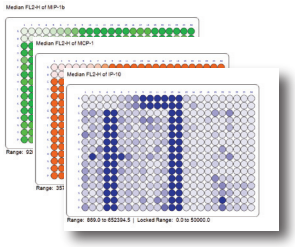
- Additional Up Time: 0.5 seconds; This specifies the amount of time the probe pauses before moving to the next well. Up time determines the spacing between samples. We recommend no adjustment.
- Pump Speed: Standard (15 RPM on Que® Standard, 29 rpm on iQue® PLUS); Specifies the rate at which samples are introduced to the detectors. The default setting of standard roughly corresponds to introduction of ~1.5 µL per second. We recommend no adjustment.
- Interwell Shake: 4 seconds at 3000 RPM after every 24 wells; Specifies the insertion of a rinse and shake after a set number of wells. The specified shake speed may vary depending on the system the assay is to be performed on. Use the default shake speed specified with the supplied template.
- Cytometer Speed: Medium; Specifies the flow rate of the detector, which affects the resolution of the data. We recommend no adjustment.
- Threshold: FSC-H at 80,000 on iQue® Standard; 100,000 on iQue® PLUS; Specifies the lower thresholds for data acquisition. The current setting on FSC will filter out sub-micron events. We recommend no adjustment

4. Once all desired protocol adjustments have been made, select RUN to acquire data.
6. During the plate read, the data will automatically populate into the pre-defined analysis template.
7. Verify that the sample data aligns with the pre-defined gating strategy, and 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.

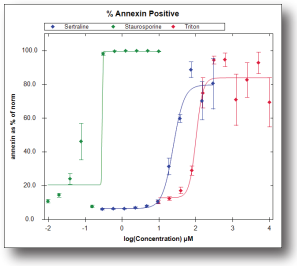
Visualization of Screening Results

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

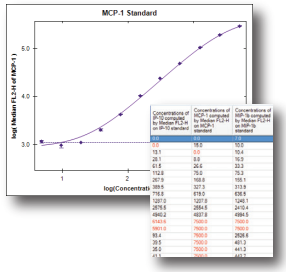


Dose Response Curves

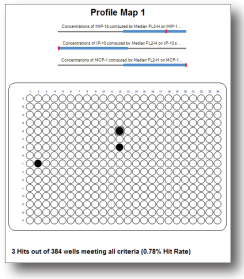


PDF Data Reports

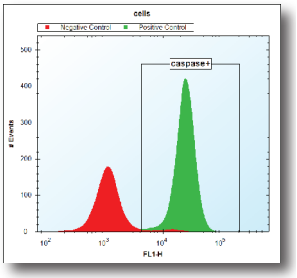
Standard Curves



Profile Maps



Overlay Plots



Appendix A: 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 5 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 B: 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 C: Worksheet for Preparing iQue Qbeads® for a Different Number of Reactions

Instructions

Each tube of iQue Qbeads® in your kit is supplied as a 50X concentrate. They must be combined and diluted to working concentration prior to use.

1. In the worksheet on the right, indicate the number of reactions you want to run in 1
2. In 2 indicate the number of analytes you are trying to detect.
3. Multiply 1 by 0.2 and record the number in 3.
4. Multiply 1 by 10 and record the number in 4.
5. Fill 5 with 4 - (2 x 3).
6. Follow the instructions in the worksheet to prepare your iQue Qbeads® for use.

Worksheet

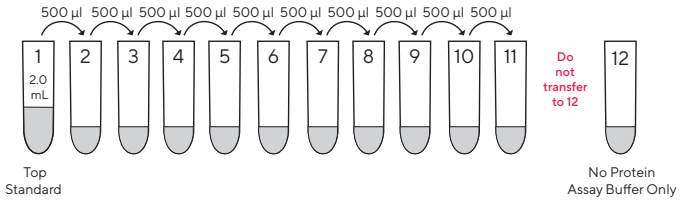
1. I want to run _____ reactions.
2. My kit is designed to detect _____ analytes.
3. Combine _____ µL of each bead.
4. The total required volume of prepared iQue Qbeads® is _____ µL.
5. Dilute combined iQue Qbeads® with _____ µL of Capture Bead Buffer.

Appendix D: Recommended Protocol for Preparation of Protein Standards

When preparing standards for quantitative iQue Qbeads® protocols, using larger transfer volumes reduces the effects of pipetting error. We recommend that titration of standards are performed in at least 500 µL volumes, and that excess standard be discarded. The following protocol is a guideline that can be adapted or optimized for each laboratory.

Before starting, ensure that all lyophilized standards have been combined and reconstituted to the proper volume. Standards require at least 15 minutes at room temperature to reconstitute before performing dilutions.

1. Prepare 12 sample tubes (microcentrifuge tubes or 12 x 75 mm FACS tubes can be used), labeled #1–12.
2. To tubes # 2–12, add 500 µL of assay buffer and temporarily set aside.
3. To tube #1, transfer at least 1 mL of the reconstituted standard. This sample is the highest concentration sample for the standard curve (top standard).
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.
5. From tube#2, transfer 500 µL volume to tube#3 and mix by pipetting. Continue transferring and mixing until you reach tube#11. Do not transfer any standard into tube#12.



6. The prepared standards can now be added to your assay plate. Transfer 10 µL of each standard to the appropriate wells of the plate. Note that you will have excess standard remaining. This standard should be used on the same day, or discarded. Saving diluted standards for future use is not recommended.

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 | HTFC® – 5 PK (makes 1)
90078	iQue® Sheath Additive Concentrate Solution | 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	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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