

# iQue Qbeads® Devscreen Streptavidin Kits

## Product Information

### Notice to Purchaser

The iQue Qbeads® Devscreen Streptavidin Kit is 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 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.

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.

### List of Catalog Numbers

Product Name		1 x 384 plate	5 x 384 plate	20 x 384 plate	50 x 384 plate
Single Plexes	QSAv1	90748	90749	90750	90751
	QSAv2	90752	90753	90754	90755
	QSAv3	90756	90757	90758	90759
	QSAv4	90760	90761	90762	90763
	QSAv5*	90764	90765	90766	90767
2-plex opt. #1	QSAv1& 3	90772	90773	90774	90775
2-plex opt. #2	QSAv2 & 4	90776	90777	90778	90779
3-plex opt. #1	QSAv 2, 3, 4	90780	90781	90782	90783
3-plex opt. #2	QSAv 1, 3, 4	90784	90785	90786	90787
4-plex	QSAv 1, 2, 3, 4	90788	90789	90790	90791
5-plex	QSAv 1, 2, 3, 4, 5	90792	90793	90794	90795

**Table 1:** List of Catalog Numbers

## Kit Contents

The iQue Qbeads® Devscreen Streptavidin Kit is comprised of streptavidin coated beads that are available in 1, 2, 3, 4 or 5-plex kits. Each kit is offered in several standard sizes with enough beads of each plex to run the specified number of 384-well plates.

Product Name	Format
iQue Qbeads® Devscreen Streptavidin Kit	1 vial (per plex ordered) 1 x 384 plate = 800 µL 5 x 384 plate = 4 mL 20 x 384 plate = 16 mL 50 x 384 plate = 40 mL

**Table 2:** iQue Qbeads® Human Devscreen Streptavidin Kit

Additional reagents required for the conjugation reaction, for QC purposes, and for downstream detection are not provided (refer to **Materials Needed but Not Provided** for complete list).







## Materials Needed but Not Provided

- Protein of interest to be coated on to beads: proteins will need to be biotinylated prior to starting this protocol
- iQue® platform
- iQue Forecyt®, versions 3.1 or higher
- Centrifuge capable of spinning microcentrifuge and | or 15 mL conical tubes at up to 8,000 g
- Centrifuge capable of spinning microplates
- Microcentrifuge tubes and | or 15 mL conical tubes
- Phosphate buffered saline (PBS) with 1% Bovine Serum Albumin (BSA) added
- Appropriate detection reagents or antibodies necessary for downstream assays with iQue Qbeads® Devscreen Streptavidin beads


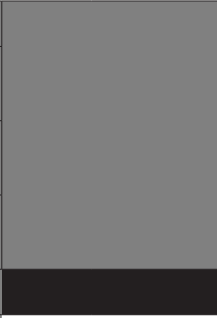





## To QC for Proper Conjugation

- PE Goat Anti-Mouse IgG Antibody (Biolegend®, Cat. No. 405307);
- PE Goat Anti-Rat IgG Antibody (Biolegend®, Cat. No. 405406);
- PE Goat Anti-Rabbit IgG Antibody (Biolegend®, Cat. No. 406421).

## Detection Channels

Detector (nm)	Spectrum	Blue Laser (488 nm)	Red Laser (640 nm)
533/30		(B/Green)	
585/40		(B/Green)	
670 LP		(B/Red)	
675/25			(R/Red) Classification Channel
780/60			(B/Red)

**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)	
572/28		(V/Yellow)	(B/Yellow)	
615/24		(V/Orange)	(B/Orange)	
675/30		(V/Red)	(B/Red)	
780/60		(V/Crimson)	(B/Crimson)	

**Table 4.** iQue® PLUS Detector Channels

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

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## Background

The binding of biotinylated substrates to streptavidin is a high affinity, chemically stable covalent interaction. iQue Qbeads<sup>®</sup> Devscreen Streptavidin beads are a family of beads that are designed for ease of use and multiplexing, enabling the application of this common chemistry to design customized assays for screening. The iQue Qbeads<sup>®</sup> Devscreen Streptavidin Kits are comprised of polystyrene beads of uniform size and excitation | emission spectra, but with spectrally-distinct fluorescence intensities that allow for bead segregation and analysis. These beads represent a unique multiplexing platform that is capable of both qualitative and quantitative analysis of multiple analytes from a single sample. In addition to being highly customizable, this panel features:

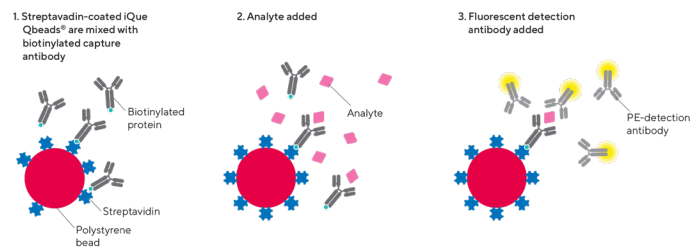
- Multiplexing with up to 5 different analytes in a single sample using the same streptavidin-biotin chemistry;
- Seamless multiplexing with other products in iQue<sup>®</sup> reagent family, including iQue Qbeads<sup>®</sup> Plexscreen products;
- Flexibility for applications such as screening of secreted proteins, fluorometric enzymatic activity analysis, protein | protein interaction, lipid | sugar binding interactions, etc.

iQue Qbeads<sup>®</sup> Devscreen Streptavidin Kit bead reagents are specifically and exclusively designed for use on iQue<sup>®</sup> platforms. These systems and the iQue Qbeads<sup>®</sup> combine to make a powerful platform that features a streamlined workflow, flexible multiplexing capabilities, and assay miniaturization.

## Assay Principles

Que Qbeads<sup>®</sup> Devscreen Streptavidin beads are a building block reagent and may be utilized in any number of different applications, depending on the type of biotinylated substrate that will be coated on the streptavidin beads.

One highlighted application is the use of streptavidin beads to build sandwich ELISAs for protein quantification. Using an example of a single analyte (**Figure A**), streptavidin beads coated with biotinylated capture antibodies directed against an analyte of interest are directly combined with samples. Samples can be from cell culture supernatant or serum. 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” (**Figure B**). The fluorescence signal is now associated with the bead complex, and the intensity of fluorescence directly correlates to the quantity of bound analyte. The streptavidin-biotin chemistry facilitates the creation of any number of custom ELISA pairs, broadly expanding the capabilities of the iQue Qbeads<sup>®</sup> family.



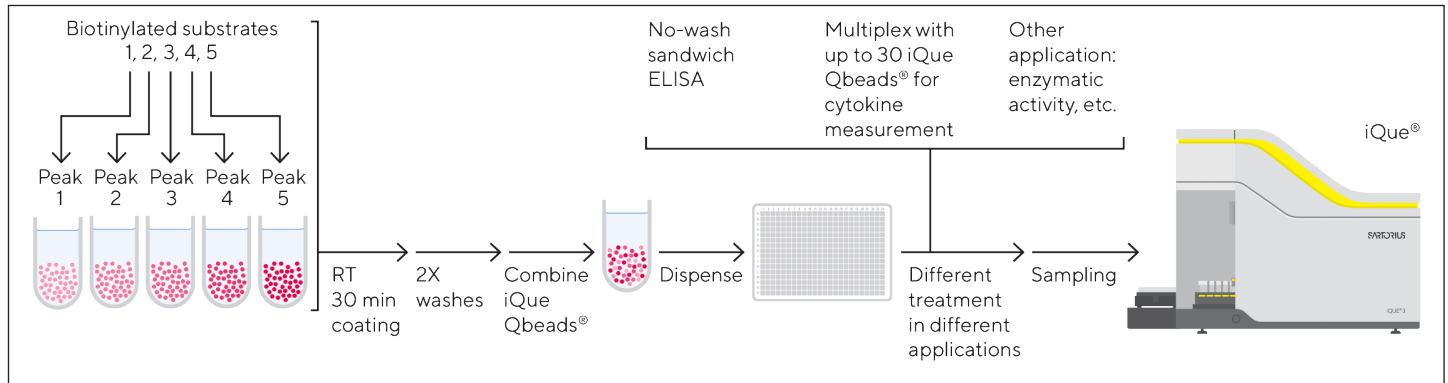
**Figure 1.** Schematic of Streptavidin-Biotin Binding Interaction on Beads. (a) Schematic of a streptavidin bead-based sandwich ELISA, showing a single streptavidin bead, with biotinylated capture antibody detecting a single analyte. Once the sandwich complex is fully formed, the fluorescence signal is directly associated with the bead, and the intensity of the signal is proportional to the amount of bound analyte.

Quantitative readouts from this assay can be measured as fluorescence intensity, or interpolated to a concentration (e.g., pg/mL) in solution via the use of a standard curve. By creating beads with differential fluorescent signatures that capture specific analytes, up to 5 analytes can be detected in a single sample, and individually identified during analysis. In addition, users may multiplex iQue Qbeads<sup>®</sup> Devscreen Streptavidin beads with iQue Qbeads<sup>®</sup> Plexscreen Secreted Protein beads, to create up to 35 plexes in a single sample.

# Workflow Overview for Batch Preparation of Beads

Streptavidin beads uniquely enable a screening friendly protocol that is ready for adaptation to various downstream applications. Coating of streptavidin beads is done in batch, and can be accomplished in

under an hour. Once the beads are conjugated with the biotinylated substrates of interest, they can be dispensed into assay plates and utilized in a no-wash analyte detection assay.



**Figure 2.** iQue Qbeads® Devscreen Streptavidin Kit Workflow on iQue® platforms. iQue Qbeads® Devscreen Streptavidin Kits are designed for a simplified workflow with ease of adaptation to various applications. Bead preparation is done in batch, by adding biotinylated substrates to the designated tubes, each with a single

population of beads. Incubate the beads at room temperature for 30 minutes, then wash 2x to remove excess reagents. Individually labeled beads are ready to be combined for downstream multiplexing experiments, and can be directly used for assays on the iQue® platform.

## Before Beginning

- Briefly centrifuge all vials before use to prevent reagent loss
- Vigorously vortex the reagents prior to use to ensure a homogenous solution.
- Ensure that sufficient quantities of all needed materials (including those that are needed but not provided) are available.
- Ensure that there is sufficient quantity of biotinylated protein for the bead conjugation reaction. Briefly centrifuge all vials before use to prevent reagent loss.

## General Protocol for Coating Biotinylated Substrates to Beads

For the following protocol, volumes are given provided for 1x 384 well plate reactions. For larger kit sizes, volumes listed should be scaled accordingly.

- 1.0 Select the appropriate streptavidin beads for your experiment.**
- 2.0 Vigorously vortex each bead vial for ~30 seconds, then transfer 675  $\mu$ L of each bead to its own labeled 1.5 mL microcentrifuge tube.**
- 3.0 Spin the tubes at 8,000 g for 3 minutes.** Carefully remove the supernatant and resuspend the beads in each tube in 675  $\mu$ L of PBS with 1% BSA.

## 4.0 Add the appropriate quantity of biotinylated substrate to each tube.

For biotinylated proteins, the final concentration of the protein should be between 1–10  $\mu$ g/mL.

## 5.0 Briefly vortex the tube to mix well.

Excessive vortexing in PBS with 1% BSA will cause foaming.

## 6.0 Incubate for 30 minutes at room temperature.

## 7.0 Add 675 $\mu$ L of PBS with 1% BSA to each tube to wash.

Spin all tubes at 8,000 g for 3 minutes. Carefully remove the supernatant.

Note: If necessary or desired, the centrifuge speed and time can be increased to better pellet beads. Care should be exercised during aspiration steps to avoid bead loss.

## 8.0 Wash beads by carefully resuspending each bead pellet in 1 mL PBS with 1% BSA.

Spin all the tubes at 8,000 g for 3 minutes. Carefully remove the supernatant.

## 9.0 Resuspend beads in a volume and buffer appropriate for downstream applications.

9.1 For multiplexing with other streptavidin coated beads, the density should be adjusted based on the number of plexes. Refer to multiplexing protocol on page 7.

9.2 For multiplexing with the iQue Qbeads® Plexscreen panel, adjust the bead density to 6 x 10<sup>6</sup> beads/mL in PBS with 1% BSA.

Note: Stock beads are provided at a density of 1 x 10<sup>6</sup> beads/mL. The volumes above are specified to create enough coated beads to support ~1500 beads per well. For preparing more or fewer reactions, the volumes can be scaled accordingly.

## Guidance for QC and Confirmation of Successful Bead Conjugation

Below are two methods used to confirm the conjugation of proteins to iQue Qbeads® Devscreen Streptavidin beads. They are provided as starting points and may need modifications to provide satisfactory results with specific applications.

### 1.0 Determining Successful Conjugation of Antibodies to Beads

- 1.1 Obtain a small aliquot (5–10 µL) of both conjugated and unconjugated beads.
- 1.2 Dilute the conjugated beads 1:50 in PBS with 1% BSA.
- 1.3 Dilute the unconjugated (control) beads 1:300 in PBS with 1% BSA.
- 1.4 Transfer 10 µL of each diluted bead to new tubes.
- 1.5 To each tube, add 10 µL of the appropriate PE-anti-Ig detector (anti-mouse or rat or rabbit, depending on species of the conjugated antibody) to the 10 µL of diluted beads.
- 1.6 Incubate at room temperature for 30 minutes, protected from light.
- 1.7 Acquire samples on your iQue® platform (transfer samples to plates if necessary)
- 1.8 The recommended criteria to confirm a successful conjugation reaction is a separation window between positive and negative signal (ie. conjugated vs. unconjugated bead) of 10 fold or greater on the PE detection (FL2 | BL2 – B/Yellow) channel.

### 2.0 Determining Successful Conjugation of Other Proteins to Beads:

- 2.1 Obtain a small aliquot (5–10 µL) of both conjugated and unconjugated beads.
- 2.2 Dilute the conjugated beads 1:50 in PBS with 1% BSA.
- 2.3 Dilute the unconjugated (control) beads 1:300 in PBS with 1% BSA.
- 2.4 Transfer 10 µL of each diluted bead to new tubes.
- 2.5 To each tube, add 10 µL of the appropriate PE-labeled anti-conjugated protein antibody to 10 µL diluted beads.  
*Note: Alternatively, biotinylated antibodies specific to the conjugated protein, followed by streptavidin-PE can be used.*
- 2.6 Incubate at room temperature for 30 minutes, protected from light.
- 2.7 Acquire samples on your iQue® platform (transfer samples to plates if necessary).
- 2.8 The recommended criteria to confirm a successful conjugation reaction is a separation window between positive and negative signal (ie. conjugated vs. unconjugated bead) of 10 fold or greater on the PE detection (FL2 | BL2 – B/Yellow) channel.

## Multiplexing Protocols: Multiplexing with other iQue Qbeads® Devscreen Streptavidin Beads

### 1.0 Prepare Capture Beads

- 1.1 After biotin-streptavidin coating is complete, resuspend beads in 250 µL of PBS with 1% BSA for each tube.
- 1.2 Obtain an accurate bead density, and then adjust the volume of each tube with additional PBS with 1% BSA according to the table below to achieve the desired bead density.

Bead Plexes	Bead density adjusted for each tube
1 plex	0.12 million per mL
2 plexes	0.24 million per mL
3 plexes	0.36 million per mL
4 plexes	0.48 million per mL
5 plexes	0.6 million per mL

- 1.3 After all individual plexes are at the appropriate density, they can be combined into a single tube. The total reaction volume should be 4.5 mL. For a single plex assay, this would be 4.5 mL of the prepared 1 plex bead. For a 2 plex assay, combine 2.25 mL from each plex into the tube, for 4.5 mL total volume. For a 5 plex assay, combine 0.9 mL of each plex into a tube, to achieve a final volume of 4.5 mL.
- 1.4 Vortex beads briefly to mix well and ensure that they form a homogenous mixture.

### 2.0 Perform the Assay

- 2.1 In each well of a 384-well plate, add 10 µL of prepared bead cocktail to 10 µL of your samples or standards.
- 2.2 Incubate at the appropriate time and temperature for your analytes.
- 2.3 Add 10 µL of detection reagent, and incubate for the appropriate time and temperature. If multiplexing, a cocktail of all the detection reagents at the appropriate dilutions should be prepared in advance.

### 3.0 Acquire Data

- 3.1 Once the incubation is complete, directly acquire data on your iQue® platform. Refer to **Page 5**.

## Multiplexing Protocols: Multiplexing with iQue Qbeads® Plexscreen Beads

The protocol listed below is equivalent to the iQue Qbeads® Plexscreen protocol. For additional protocol options, including Reduced Background and Standard Curve variants, please refer to the iQue Qbeads® Plexscreen Secreted Protein Assays manual.

### Additional Materials Needed but not Provided for Multiplexed Experiments with iQue Qbeads® Plexscreen Beads on iQue® Standard Platforms

- Selectable Detector Set (SDS) software license\*
- Alternate Emission Filter (780/60 nm) in B/Red position\*
- Plate washer if performing “Reduced Background” Protocols

\* Available in the iQue Qbeads® Enabler Bundle, Cat. No. 90331.

#### 1.0 Prepare Capture Beads

- 1.1 After biotin-streptavidin coating is complete, resuspend beads in 50 µL of PBS with 1% BSA for each tube.
- 1.2 Obtain an accurate bead density, and then adjust the volume of each tube with additional PBS with 1% BSA to achieve a density per plex of  $6 \times 10^6$  beads/mL (50x).
- 1.3 Vigorously vortex the capture beads for each analyte, including streptavidin coated and iQue Qbeads® Plexscreen beads.
- 1.4 In a 15 mL conical tube, combine 90 µL of beads for each analyte.
- 1.5 Bring the final volume of the bead mixture to 4.5 mL with capture bead buffer (provided in iQue Qbeads® Plexscreen kit). For samples utilizing serum or plasma, ensure that the capture bead buffer for serum | plasma is used. All other sample types should utilize the standard capture bead buffer. Note that the amount of buffer added will vary according to the number of plexes in the assay.
- 1.6 Vortex beads briefly to mix well and ensure that they form a homogenous mixture.

The volumes here are to prepare enough reagent for 1 x 384 well plate with minimal overage. To prepare reagent for partial plates or with more overage, refer to **Appendix C**.

#### 2.0 Prepare Detection Reagent

The detection reagents for the iQue Qbeads® Devscreen Streptavidin beads will need to be sourced and characterized individually to determine optimal concentrations and performance. This characterization and assay development will need to be done in advance of multiplexing.

The detection antibodies in the iQue Qbeads® Plexscreen kit are provided pre-mixed, and at working concentration. For optimal performance in this assay, select a detection reagent that can be utilized with AT LEAST a 1:100 dilution factor to minimize the impact to the Plexscreen detection reagent concentrations.

- 2.1 Create a detection reagent cocktail for all the analytes to be detected by diluting the streptavidin beads detection reagents directly into the Plexscreen detection cocktail. Note that this step will in effect dilute out the Plexscreen detection cocktail, and steps should be taken to minimize the dilution.
- 2.2 Mix gently and protect from light until use.

#### 3.0 Capture Analytes onto Beads

- 3.1 In each well of a 384-well plate, add 10 µL of prepared beads to 10 µL of your samples or standards. Ensure that all samples are at the bottom of the plate and are not adhered to the side walls of the well.
- 3.2 Mix the plate using a plate shaker and ensure thorough mixing. The shaker on your iQue® platform can be utilized for this step. Refer to **Appendix A** for appropriate shake speeds.
- 3.3 Incubate the plate with lid on at room temperature for 1 hour, protected from light.

#### 4.0 Add Detection Reagent

- 4.1 Add 10 µL of detection reagent to each well. Ensure that all samples are at the bottom of the plate and are not adhered to the side walls of the well.
- 4.2 Mix the plate using a plate shaker and ensure thorough mixing. The shaker on your iQue® platform can be utilized for this step. Refer to **Appendix A** for appropriate shake speeds.
- 4.3 Incubate the plate with lid on at room temperature for 2 hours, protected from light.

## 5.0 Acquire Data

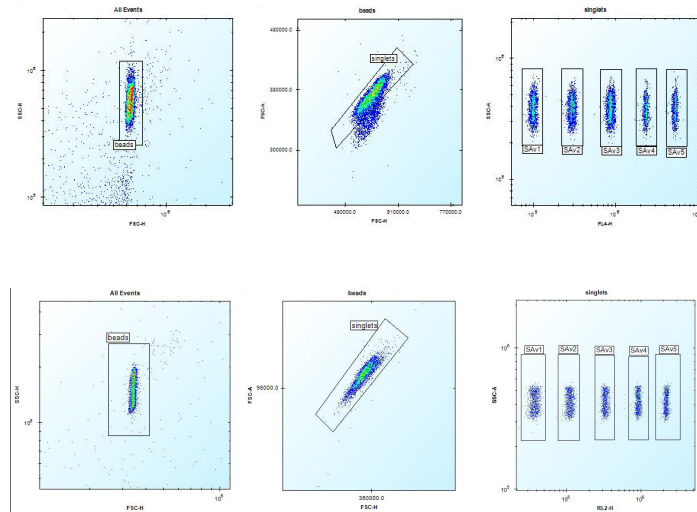
1. Ensure that the iQue® platform is in the 2:2 SDS Configuration and the appropriate filter is installed.
2. Once the incubation is complete, directly acquire data on your iQue® platform.

On standard iQue® platforms, 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® Plexscreen 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 modifications are necessary if utilizing this kit on the iQue® PLUS platform.

## 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 your kit. The template provided is not unique to a specific plex size and will represent all 5 potential plexes. For any plexes not utilized in the experiment, delete the gate for that plex.

The template contains a preset sampling protocol for data acquisition and provides automated identification of the plexes in your sample. Note that any downstream analyses will need to be defined for the specific detection reagents used.



**Figure 3.** Template for DevScreen Streptavidin Coated Beads. A template is provided with your kit that predefines an analysis for the 5 different peaks of streptavidin beads. Shown above are gates that identify bead populations. Before acquisition, the bead population gates will be empty. During acquisition, the gates will populate with the appropriate beads as they pass through your screening system's detector. In the unlikely event that a gate does not completely encompass a bead population, click the gate label and drag to shift the gate. Data shown are for the iQue® Standard (top) and iQue® PLUS (bottom).

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>.

3. The default acquisition settings 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.

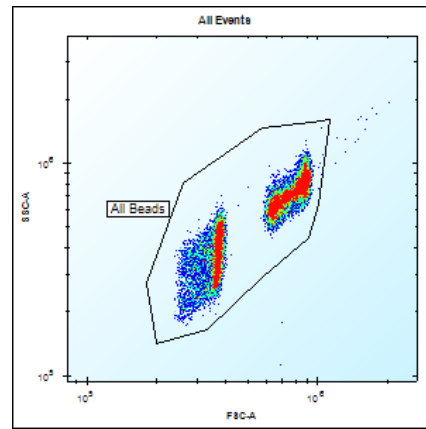
- **Pre-Plate Prime:** 60 seconds; Prior to sampling a plate, the system will prime the tubing with the S1 rinse station buffer for the specified amount of time. The time can be decreased if a faster acquisition is desired and | or tubing is already clean. Increasing the prime time is not recommended.
- **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.

If screening dose response series on your plate, we recommend sampling from low to high concentration. Additionally, we recommend an interwell shake after the high dose in each series to minimize any potential signal carry-over to lower dose wells. See below for the recommended interwell shake parameters.

- **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  $\mu$ L per second. We recommend no adjustment.

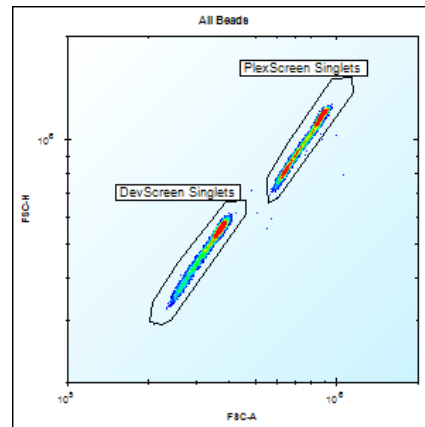


- **Interwell Shake:** 4 seconds at 3000 RPM after every 24 wells; Specifies the insertion of a rinse and shake after a set number of wells. The specified shake speed may vary depending on the system the assay is to be performed on. Use the default shake speed specified with the supplied template. The frequency of shake can be adjusted after any number of wells to better accommodate the sample layout on the plate
  - **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.



## 2.0 Rename the existing singlet gate to “Plexscreen Singlets.”

If necessary, move the gate. Create a new singlet gate for the “Devscreen SAv Singlets” for the beads on the left as shown.



## Templates for Multiplexing with iQue Qbeads® Plexscreen

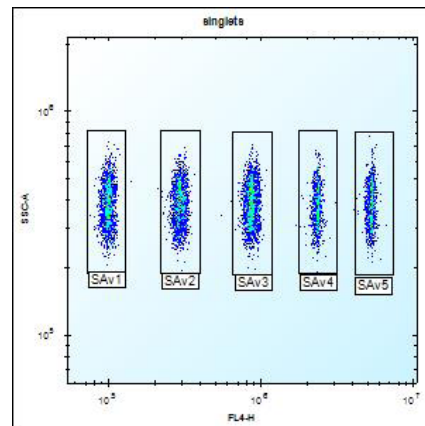
For experiments multiplexing with iQue Qbeads® Plexscreen beads – it is recommended to utilize the customized template that was provided with your Plexscreen kit, and add in the gating strategy for the iQue Qbeads® Devscreen Streptavidin beads. The gates that will need to be adjusted are as follows:

### 1.0 Expand the All Beads gate to include the iQue Qbeads® Devscreen Beads as shown.

The iQue Qbeads® Devscreen and iQue Qbeads® Plexscreen beads are different sizes, with the iQue Qbeads® Devscreen beads being smaller and appearing to the left of the iQue Qbeads® Plexscreen beads.

### 3.0 Gate the Devscreen SAv singlets on an FL4-H histogram (iQue® Standard) or RL2-H histogram (iQue® PLUS)

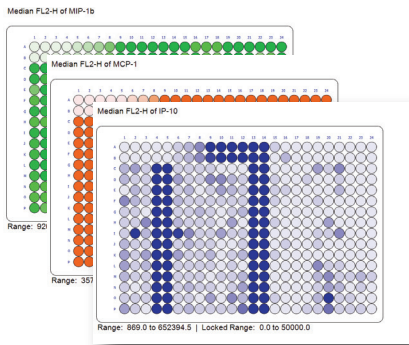
Each peak will represent a single plex.



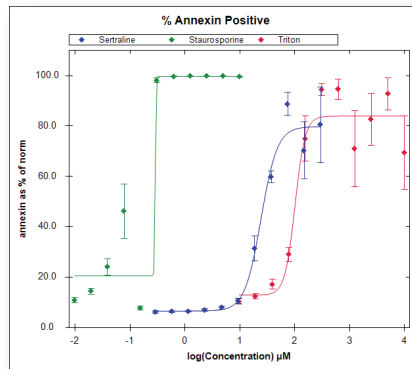
# Visualization of Screening Results

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](http://www.intellicyt.com/resources).

## Heat Maps



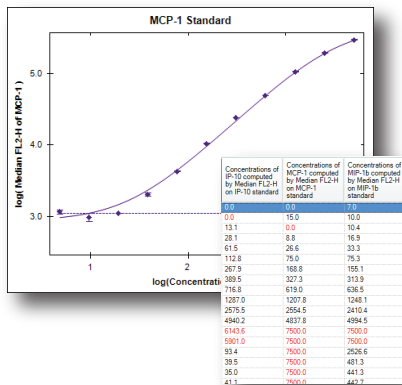
## Dose Response Curves



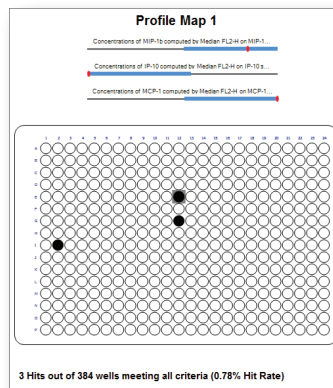
## PDF Data Reports



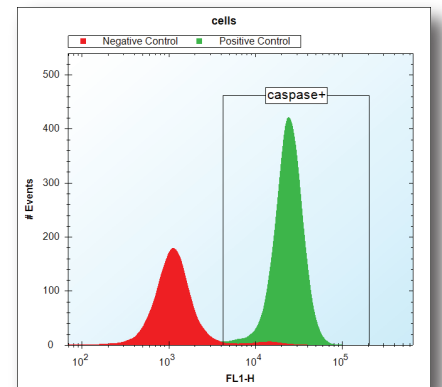
## Standard Curves



## 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

When determining the number of reactions to prepare reagents for, it is recommended to increase the calculated reactions by ~10% to account for some overage in pipetting. (ie. rather than preparing reagent for exactly 96 reactions, prepare enough reagent for 110 reactions to accommodate small pipetting discrepancies).

The reaction calculations assume that all beads have been density adjusted to  $6 \times 10^6$  beads/mL density for multiplexing with iQue Qbeads® Plexscreen kits.

Instructions	Worksheet
<p>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.</p> <p>1. In the worksheet on the right, indicate the number of reactions you want to run in <b>1</b>.</p> <p>2. In <b>2</b>, indicate the number of analytes you are trying to detect.</p> <p>3. Multiply <b>1</b> by 0.2 and record the number in <b>3</b>.</p> <p>4. Multiply <b>1</b> by 10 and record the number in <b>4</b>.</p> <p>5. Fill <b>5</b> with <b>4</b> - (<b>2</b> x <b>3</b>).</p> <p>6. Follow the instructions in the worksheet to prepare your iQue Qbeads® for use.</p>	<p><b>1</b> I want to run _____ reactions.</p> <p><b>2</b> My kit is designed to detect _____ analytes.</p> <p><b>3</b> Combine _____ <math>\mu</math>L of each bead.</p> <p><b>4</b> The total required volume of prepared iQue Qbeads® is _____ <math>\mu</math>L.</p> <p><b>5</b> Dilute combined iQue Qbeads® with _____ <math>\mu</math>L of Capture Bead Buffer.</p>

# 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](http://www.sartorius.com/iQue) for complete list. Contact your local area sales representative for part number and pricing information

## Sales and Service Contacts

For further contacts, visit  
[www.sartorius.com](http://www.sartorius.com)

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