SARTORIUS

Product Guide

iQue Qbeads® Devscreen SH Kits

Product Information

Notice to Purchaser

The iQue Qbeads® Devscreen SH 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.

Kit Contents

The iQue Qbeads® Devscreen SH Reagent Panel is comprised of 30 individual beads, each sold individually. Each kit provides enough reagent to prepare beads for 50 x 384-well plates. Protocols for both full (50 x 384) and partial reactions (5 x 384 wells) are provided. Listed part numbers for each singleplex come with coupling buffer and storage buffer required for conjugation.

Product Name	Format
iQue QBeads® Devscreen SH Bead	1 vial (per plex ordered)
iQue® S1 Fluid Station QSol Buffer Cartridge	1 bottle, 150 mL (1x)
P200 Pipet Tip for Puncturing Cartridge	1 bottle, 90 mL (1x)

Table 1: Kit Contents

Note: Additional reagents required for the conjugation reaction, for QC purposes, and for downstream detection are not provided (refer to "Materials Needed but Not Provided" page iv for complete list)

List of Catalog Numbers

Product Name	Cat. No.	Storage
iQue Qbeads® Devscreen SH Kits QSH01 50 x 384 plate: Coupling and Storage Buffers, Beads	90892	2–8 °C
iQue Qbeads® Devscreen SH Kits QSH02 50 x 384 plate: Coupling and Storage Buffers, Beads	90893	2–8 °C
iQue Qbeads® Devscreen SH Kits QSH03 50 x 384 plate: Coupling and Storage Buffers, Beads	90894	2–8 °C
iQue Qbeads® Devscreen SH Kits QSH04 50 x 384 plate: Coupling and Storage Buffers, Beads	90895	2–8 °C
iQue Qbeads® Devscreen SH Kits QSH05 50 x 384 plate: Coupling and Storage Buffers, Beads	90896	2–8 °C
iQue Qbeads® Devscreen SH Kits QSH06 50 x 384 plate: Coupling and Storage Buffers, Beads	90897	2–8 °C
iQue Qbeads® Devscreen SH Kits QSH07 50 x 384 plate: Coupling and Storage Buffers, Beads	90898	2–8 °C
iQue Qbeads® Devscreen SH Kits QSH08 50 x 384 plate: Coupling and Storage Buffers, Beads	90899	2–8 °C
iQue Qbeads® Devscreen SH Kits QSH09 50 x 384 plate: Coupling and Storage Buffers, Beads	90900	2–8 °C
iQue Qbeads® Devscreen SH Kits QSH10 50 x 384 plate: Coupling and Storage Buffers, Beads	90901	2–8 °C
iQue Qbeads® Devscreen SH Kits QSH11 50 x 384 plate: Coupling and Storage Buffers, Beads	90902	2–8 °C
iQue Qbeads® Devscreen SH Kits QSH12 50 x 384 plate: Coupling and Storage Buffers, Beads	90903	2–8 °C
iQue Qbeads® Devscreen SH Kits QSH13 50 x 384 plate: Coupling and Storage Buffers, Beads	90904	2–8 °C
iQue Qbeads® Devscreen SH Kits QSH14 50 x 384 plate: Coupling and Storage Buffers, Beads	90905	2–8 °C
iQue Qbeads® Devscreen SH Kits QSH15 50 x 384 plate: Coupling and Storage Buffers, Beads	90906	2–8 °C
iQue Qbeads® Devscreen SH Kits QSH16 50 x 384 plate: Coupling and Storage Buffers, Beads	90907	2–8 °C
iQue Qbeads® Devscreen SH Kits QSH17 50 x 384 plate: Coupling and Storage Buffers, Beads	90908	2–8 °C
iQue Qbeads® Devscreen SH Kits QSH18 50 x 384 plate: Coupling and Storage Buffers, Beads	90909	2–8 °C
iQue Qbeads® Devscreen SH Kits QSH19 50 x 384 plate: Coupling and Storage Buffers, Beads	90910	2–8 °C
iQue Qbeads® Devscreen SH Kits QSH20 50 x 384 plate: Coupling and Storage Buffers, Beads	90911	2–8 °C
iQue Qbeads® Devscreen SH Kits QSH21 50 x 384 plate: Coupling and Storage Buffers, Beads	90912	2–8 °C
iQue Qbeads® Devscreen SH Kits QSH22 50 x 384 plate: Coupling and Storage Buffers, Beads	90913	2–8 °C
iQue Qbeads® Devscreen SH Kits QSH23 50 x 384 plate: Coupling and Storage Buffers, Beads	90914	2–8 °C
iQue Qbeads® Devscreen SH Kits QSH24 50 x 384 plate: Coupling and Storage Buffers, Beads	90915	2–8 °C
iQue Qbeads® Devscreen SH Kits QSH25 50 x 384 plate: Coupling and Storage Buffers, Beads	90916	2–8 °C
iQue Qbeads® Devscreen SH Kits QSH26 50 x 384 plate: Coupling and Storage Buffers, Beads	90917	2–8 °C
iQue Qbeads® Devscreen SH Kits QSH27 50 x 384 plate: Coupling and Storage Buffers, Beads	90918	2–8 °C
iQue Qbeads® Devscreen SH Kits QSH28 50 x 384 plate: Coupling and Storage Buffers, Beads	90919	2–8 °C
iQue Qbeads® Devscreen SH Kits QSH29 50 x 384 plate: Coupling and Storage Buffers, Beads	90920	2–8 °C
iQue Qbeads® Devscreen SH Kits QSH30 50 x 384 plate: Coupling and Storage Buffers, Beads	90921	2–8 °C

Table 2: List of Catalog Numbers

Detection Channels

Detector (nm)	Spectrum	Blue Laser (488 nm)	Red Laser (640 nm)	
533/30		(B/Green)	SDS 2:2	
585/40		(B/Yellow)		
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)		
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

- Protein of interest to be coated on to beads: proteins should be in carrier-free formulations, and adjusted to 1 mg/mL in PBS
- iQue® platform
- 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)
- 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 FACS tubes
- Centrifuge capable of spinning microplates
- Orbital shaker
- 12 x 75 mm FACS tubes (minimum quantity 2 for partial reaction; minimum quantity 20 for full reaction
- Microcentrifuge tubes and | or 15 mL conical tubes
- Phosphate buffered saline (PBS) with 1% Bovine Serum Albumin (BSA)
- Deionized (DI) water
- DTT powder; Pierce® Cat. No. 20290 (can be pre-made | aliquoted | stored at -20°C; 1 M DTT, prepared in DI water)
- Lyophilized Sulfo-SMCC; Pierce® Cat. No. 22322 (prepare fresh immediately before use, 2 mg/mL in DI water)
- N-Ethylmaleimide (NEM); Pierce® Cat. No. 23030 (can be pre-made | aliquoted | stored at -20°C; 2 mg/mL in DMSO)
- Buffer exchange columns Bio-spin P30; Bio-Rad Cat. No. 732-6231 (quantity 25) or Cat. No. 732-6232 (quantity 100)

  Note: 1 column is needed for each partial reaction; 10 columns are needed for a full reaction.

To QC for Proper Conjugation

For antibody conjugations, we recommend the following:

- 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

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Background

The iQue Qbeads® Devscreen SH beads are a panel of reagents that are designed to enhance the flexibility, ease of use, and multiplexing options of the iQue Qbeads® product line. iQue Qbeads® Devscreen SH beads rely on a common sulfhydryl chemistry to enable the design of customized beads for screening. Customers covalently attach proteins of interest such as capture antibodies to the derivatized beads. In addition to being highly customizable, this panel features downstream assay benefits including:

- Multiplexing up to 30 different analyte measurements in a single well;
- Seamless multiplexing with other products in iQue® reagent family, including iQue Qbeads® Plexscreen kits and iQue Qbeads® Devscreen SAv products;
- Flexibility for applications such as screening of secreted proteins, fluorometric enzymatic activity analysis, protein | protein interaction, lipid | sugar binding interactions, etc.

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

Assay Principles

The iQue Qbeads® Devscreen SH bead panel is comprised of polystyrene beads of uniform size and excitation | emission spectra, but with spectrally-distinct fluorescence intensities that allow for bead segregation and analysis. iQue Qbeads® Devscreen SH beads are a building block reagent and may be utilized in any number of different applications, depending on the type of substrate that will be coated on the beads.

One highlighted application is the use of iQue Qbeads® Devscreen SH beads to build sandwich ELISAs for analyte quantification. The functionalized sulfhydryl chemistry facilitates the creation of any number of bead-based ELISA assays, broadly expanding the capabilities of the iQue Qbeads® family. A schematic workflow, from conjugation to use in an assay for a single analyte is shown in **Figure 1**. The iQue Qbeads® Devscreen SH beads are first activated then conjugated with capture antibodies. Prepared beads are directly combined with samples. Fluorescent detection antibodies are used to quantify the bound analyte, with the intensity of fluorescence correlating to the quantity of bound analyte.

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.

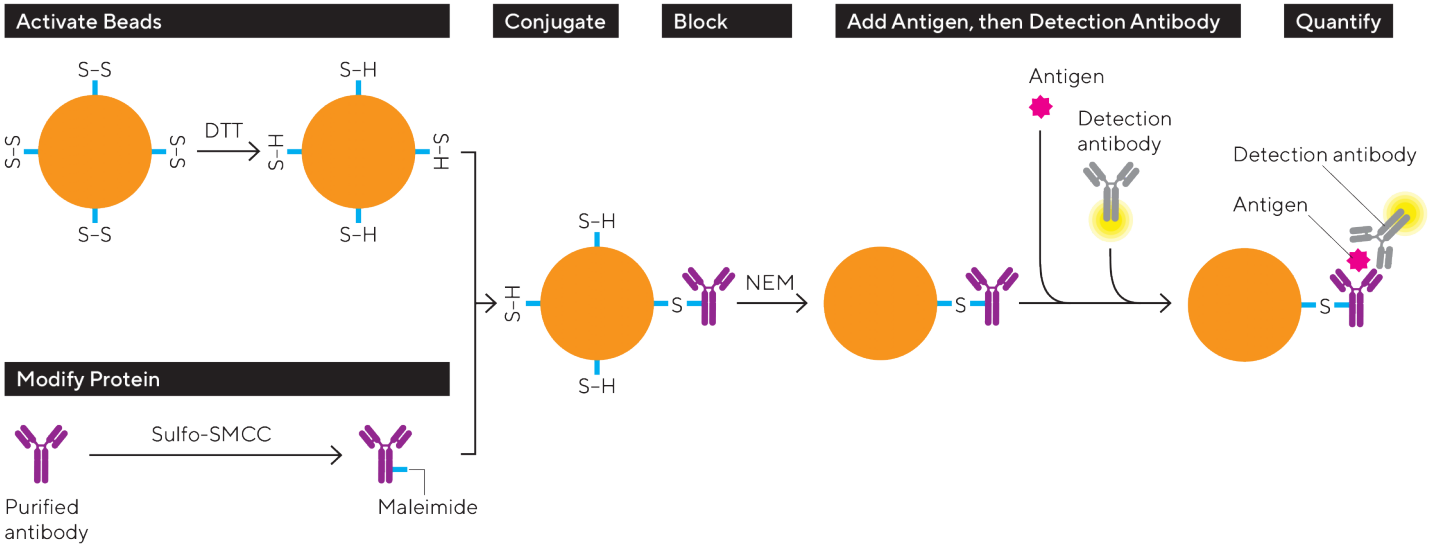


Figure 1. Schematic of an iQue Qbeads® Devscreen SH bead-based ELISA workflow. A single SH bead population is activated and then conjugated with an SMCC-modified antibody. The addition of sample (containing analyte) and detection reagent completes the sandwich (the intensity of the detection signal is proportional to the amount of bound analyte) and is read on an iQue® platform.

Workflow: Batch Preparation of Beads

The iQue Qbeads® Devscreen SH Kit uniquely enables a screening friendly protocol that is ready for adaptation to various applications. Coating of iQue Qbeads® Devscreen SH beads is done in batch, and can be accomplished in under 4 hours. Once the beads are conjugated with the substrates of interest, they can be dispensed into assay plates and utilized in a no-wash analyte detection assay.

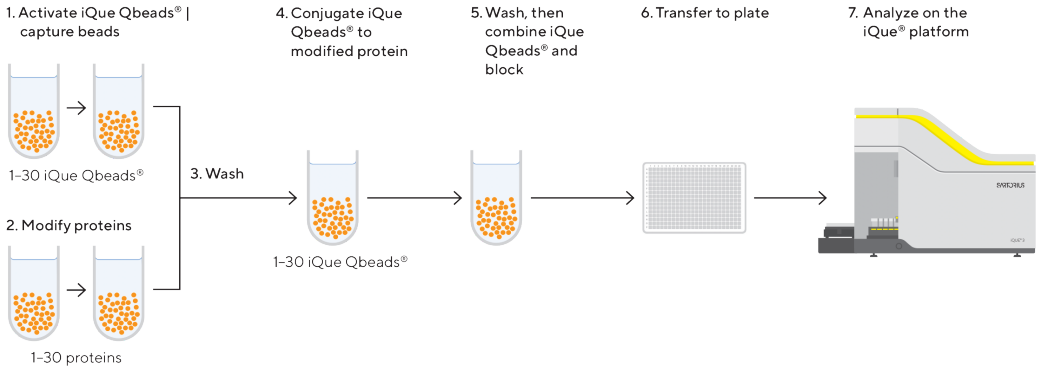


Figure 2. iQue Qbeads® Devscreen SH Kit workflow on iQue® platform. iQue Qbeads® Devscreen SH beads are designed for a simplified workflow with ease of adaptation to various applications. Bead preparation is done in batch, by adding DTT to the designated bead tubes, each with a single population of beads. Incubate the beads at room temperature for 1 hour, then wash to remove excess reagents. Modification of the capture protein is also done in batch, by adding sulfo-SMCC to the designated protein tubes, each with a single protein type. Incubate the protein at room temperature for 1 hour, and then utilize a column to exchange unbound reagents. Once preparatory steps are complete, modified proteins and activated beads are directly combined. After an hour long conjugation reaction, NEM is added to block the remaining active sites on the beads. 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.
- Ensure that all needed materials and sufficient quantities of buffer exchange columns are available. Refer to page v for a complete list of materials that are needed but not provided with this kit.
- Ensure that the protein sample to be coated is free of BSA, glycine, Tris or any other proteins or protein stabilizing additives. The protein must be suspended in PBS (pH 7.2) at 1 mg/mL concentration for successful coupling to the beads.
- Ensure that there is sufficient quantity (90 µL or 900 µL for 5 x 384 or 50 x 384 respectively) of 1 mg/mL protein in PBS for bead conjugation reaction.

General Protocol for Coating Protein Substrates to Beads

Note: For the following protocol, volumes are given for both 5 x 384 wells and 50 x 384 wells (in parentheses). Use the volume appropriate to the scale of conjugation you are performing.

1.0 Activate Beads

1.1 Select and obtain the appropriate iQue Qbeads® Devscreen SH beads for your experiment.

1.2 Vigorously vortex each bead vial for ~30 seconds, then transfer 75 µL (750 µL for 50 x 384) of each bead to its own labeled 1.5 mL (15 mL for 50 x 384) micro-centrifuge tube.

1.3 Add 1.9 µL (19 µL for 50 x 384) of 1M DTT to each tube, vortex to mix well. Incubate at room temperature for 1 hour on an orbital shaker (~150 rpm). If a shaker is unavailable, vortex the beads every 15 minutes. Protect beads from light during this incubation.

1.4 Add 1 mL (10 mL for 50 x 384) of coupling buffer to the beads, pellet beads by centrifugation at 8,000 g for 3 minutes. Carefully aspirate the supernatant, taking care to not disturb the bead pellet.

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

1.5 Resuspend the beads in 1 mL (10 mL for 50 x 384) of coupling buffer.

1.6 Repeat **Steps 1.4 and 1.5** twice more to wash the beads.

1.7 After the final wash, resuspend the beads in 20 µL (200 µL for 50 x 384) of coupling buffer.

1.8 Protect the activated beads from light and set them aside for the following steps. You will not need beads during the following protein modification steps.

2.0 Modify Proteins

2.1 Obtain a new tube for the following protein modification steps.

2.2 Transfer 90 µL (900 µL for 50 x 384) of 1 mg/mL carrier-free protein to a 1.5 mL (15 mL for 50x384) micro-centrifuge tube.

2.3 Add 2 µL (20 µL for 50 x 384) of freshly prepared sulfo-SMCC (refer to **Before Beginning** section) to the protein solution. Gently mix the solution. Do not vortex. Incubate the reaction at room temperature for 1 hour on an orbital shaker (150 RPM). If a shaker is unavailable, gently agitate the solution every 15 minutes.

Note: Adherence to the 1 hour incubation time is critical. Do not over or under incubate this reaction.

1.4 During the incubation, begin priming the buffer exchange column (see below); this step takes ~35 minutes.

1.5 After incubation is complete, directly proceed to buffer exchange step.

3.0 Prime the Buffer Exchange Column

3.1 Prime the Bio-spin P30 buffer exchange column(s) by adding 1.5 mL of coupling buffer per column. A 5 x 384 sized reaction will require one column, and a 50 x 384 sized reaction will require a total of 10 columns.

3.2 Allow the columns to drain by gravity. Repeat the priming step twice. Note that the total process of priming will require ~35–40 minutes, and it is highly recommended that this step be done while the protein modification reaction is incubating.

3.3 Once columns have been primed, transfer each column to a 12 x 75 mm tube (aka 5 mL FACS tube). Spin the column(s) inside the 12 x 75 mm test tube at 1000 g for 2 minutes to remove any residual buffer. It is important that 12 x 75 mm tubes be used so that the column is held upright with sufficient dead space below the column to receive the buffer during the centrifugation step.

3.4 Transfer the primed and dried column(s) to new 12 x 75mL test tubes.

4.0 Perform Buffer Exchange on Modified Proteins

4.1 Immediately after the 1 hour incubation for protein modification, transfer 90 µL of the protein | sulfo-SMCC solution to each prepared buffer exchange column. Note that for a 5 x 384 reaction, the total volume will be transferred to a single column. For a 50 x 384 reaction, the total volume should be equally divided to 10 different columns.

4.2 Ensure that each column has been placed inside a clean and empty 12 x 75 mm tube. Centrifuge the columns at 1000g for 2 minutes.

4.3 Visually verify that the entirety of the reaction volume has flowed through the column. Gently remove and discard the columns.

4.4 Collect and combine into a single tube (if appropriate) the volume that has flowed through the column as that fraction will contain the modified proteins. Unreacted reagents will be held inside the column.

5.0 Conjugate Proteins to Beads

5.1 Carefully transfer the modified protein (from **Step 4.0**) to the tube containing activated beads (from **Step 1.0**). Vortex briefly to mix well. Incubate the tube at room temperature for 1 hour on orbital shaker (150 RPM), protected from light.

5.2 Add 2 µL (20 µL for 50 x 384) of 2 mg/mL NEM to the bead | protein mixture. Vortex briefly to mix well. Incubate the tube at room temperature for 15 minutes on an orbital shaker (150 RPM), protected from light.

5.3 Add 1 mL (10 mL for 50 x 384) of storage buffer to the beads, and pellet beads by centrifugation at 8,000 g for 3 minutes. Carefully aspirate the supernatant, taking care to not disturb the bead pellet.

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

5.4 Resuspend the conjugated beads in 1 mL (10 mL for 50 x 384) of storage buffer.

5.5 Repeat **Steps 3 – 4** twice more to wash the beads.

5.6 After the final wash, resuspend the conjugated beads in 100 µL (1.0 mL for 50 x 384) of storage buffer.

5.7 Determine bead count by hemacytometer or other appropriate method, and adjust the bead density with storage buffer as necessary. For standard protocols and for plexing with iQue® reagent kits, we recommend a final density of $6x10^6$ beads/mL.

5.8 Store beads at 2–8°C, protected from light, for future use. Stability of the conjugated beads will vary and will need to be determined for each specific antigen or protein coated.

Guidance for QC and Confirmation of Successful Bead Conjugation

Below are two methods used to confirm the conjugation of proteins to iQue Qbeads® Devscreen SH 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 conjugated beads 1:50 in PBS with 1% BSA.

1.3 Dilute 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 (B/Yellow) | 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 conjugated beads 1:50 in PBS with 1% BSA.

2.3 Dilute 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 (B/Yellow) | BL2 (B/Yellow)) channel.

Data Acquisition and Analysis

1. Launch iQue Forecyt® (version 3.1 or greater).
2. Ensure that the iQue® platform is licensed for the Selectable Detector Set (SDS) option. Configure the SDS to the 2:2 detection setting by following the wizard and prompts in iQue Forecyt®. Create a new experiment using the standard assay template provided on the USB flash drive in the kit.

   The template provided is not unique to the specific plex size or analyte set, and will contain all 30 potential bead positions. The user will need to identify and rename the bead positions that are used in the current assay, and delete the extra bead gates that are not being used.

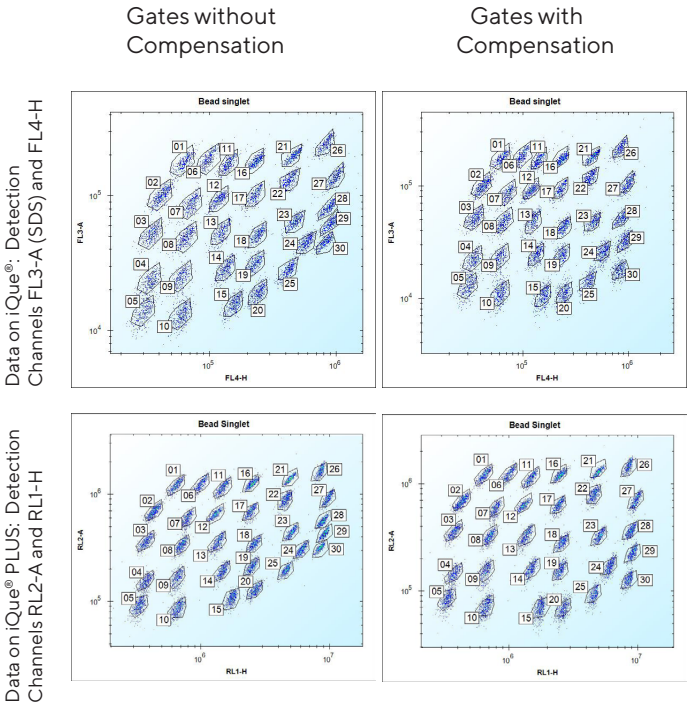


Figure 3. A template is provided with each kit that predefines an analysis for all 30 potential bead positions, and users will need to remove gates not in use for the specific experiment. Before acquisition, the bead population gates will be empty. During acquisition, the gates will populate with the appropriate beads as they are read. In the unlikely event that 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.

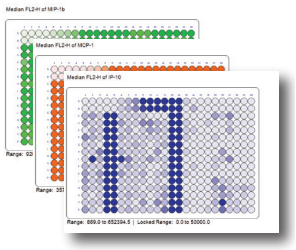
Additionally, an interwell shake is recommended after the high dose in each series to prevent any potential signal carry-over to lower dose wells. The shaking interval on the protocol should be adjusted for the specific number of wells in each dose series.

3. The default acquisition settings in bold are briefly explained below. 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 shake speed is specified assuming that the sample contains ~30 µL of total sample volume. For samples that contain significantly higher volumes, the shake speed will need to be decreased to prevent samples from spilling out of the well.
   - 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 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.
5. During the plate read, the data will automatically populate into the pre-defined analysis template.
6. 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.

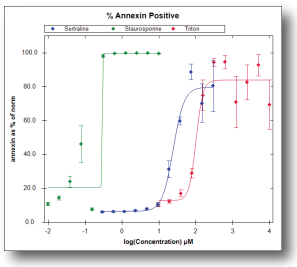
Visualization of Screening Results

After all the gates have been verified and adjusted as necessary for the plate-level data set, additional analyses including heat maps, dose responses, and standard curves can be generated (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.sartorius.com/en/products/flow-cytometry/flow-cytometry-software.

Heat Maps

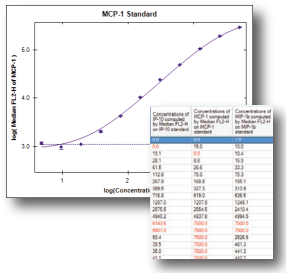


Dose Response Curves

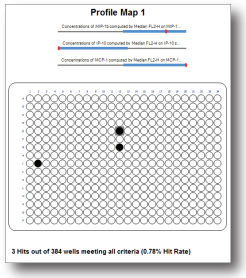


PDF Data Reports

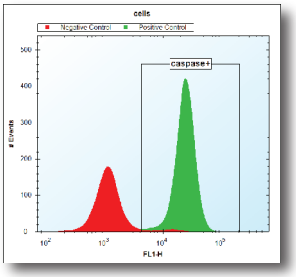
Standard Curves



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

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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