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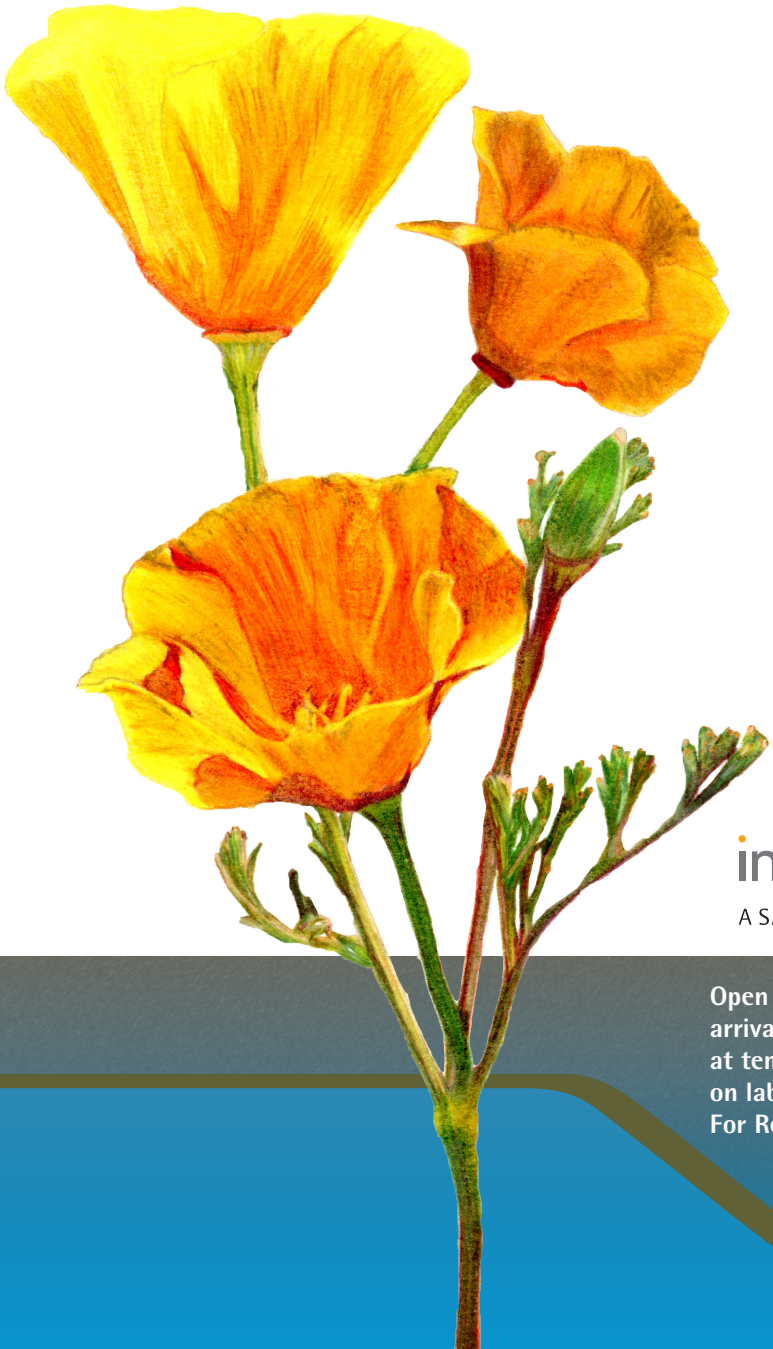
Cy-Clone PLUS Kit



intellicyt®
A SARTORIUS BRAND

Open immediately upon arrival and store reagents at temperatures stated on labels.
For Research Use Only.

Notice to Purchaser
The Cy-Clone™ PLUS Kit is a member of the IntelliCyt product line that has been extensively tested for live cell analysis applications. These screening kits are validated as complete screening assays and are optimized for use in high content screening applications. IntelliCyt's building blocks and reagents are designed for flexibility in multiplexing and incorporation into screening assays. IntelliCyt reagent kits are specifically formatted for optimal performance on IntelliCyt Screening platforms.

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

Trademarks and Patents
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List of Catalog Numbers

Description	Catalog No
Cy-Clone PLUS Kit 1x384 wells	91142
Cy-Clone PLUS Kit 5X384 wells	91143
Cy-Clone PLUS Kit 20X384 wells	91144
Cy-Clone PLUS Kit 50x384 wells	91145

Kit Contents

Component	Quantity Provided
IgG Capture Beads	1 vial
Human FITC-IgG	1 vial
Control Human IgG	1 vial
FL4 Cell Membrane Integrity Reagent	1 vial
Sample Reaction Buffer**	1 bottle
BSA (lyophilized)**	1 bottle

NOTE: BSA should be added to the Sample Reaction Buffer before use.

Detection Channels

iQue Screener **Standard** Detector Channels					
Detector	**Spectrum**	**Blue Laser (488 nm)**		**Red Laser (640 nm)**	
533/30 nm		FL1	Bead Detection Channel		
585/40 nm		FL2			
670 nm LP		FL3			
675/25 nm				FL4	Cell Viability Detection Channel

iQue Screener **PLUS** Detector Channels							
Detector	**Spectrum**	**Violet Laser (405 nm)**		**Blue Laser (488 nm)**		**Red Laser (640 nm)**	
445/45 nm		VL1					
530/30 nm		VL2		BL1	Bead Detection Channel		
572/28 nm		VL3		BL2			
615/24 nm		VL4		BL3			
675/30 nm		VL5		BL4		RL1	Cell Viability Detection Chanel
780/60 nm		VL6		BL5		RL2	

Materials Needed but Not Provided

- IntelliCyt Screening System
- ForeCyt® Software
- 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
- Complete cell culture media

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Introduction

A key component of the development of therapeutic monoclonal antibodies is the identification and selection of a manufacturing cell line that secretes high levels of antibody into the culture supernatant. Early identification of high producing cell lines can significantly increase the probability of success in downstream scale up activities.

The Cy-Clone PLUS Kit is a multiplexed assay for the IntelliCyt Screening platforms that simultaneously reports on IgG quantity, cell number and cell viability in each well for cell line production screening plates. These metrics enable the precise calculation of the IgG quantity per cell and per viable cell to better inform on cell productivity.

The Cy-Clone PLUS Kit was designed for ease of use in multiplexing, enabling a straightforward workflow without sacrificing assay performance. Compared to other IgG quantitation methods, the IntelliCyt solution offers several unique advantages:

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

Assay Principles

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

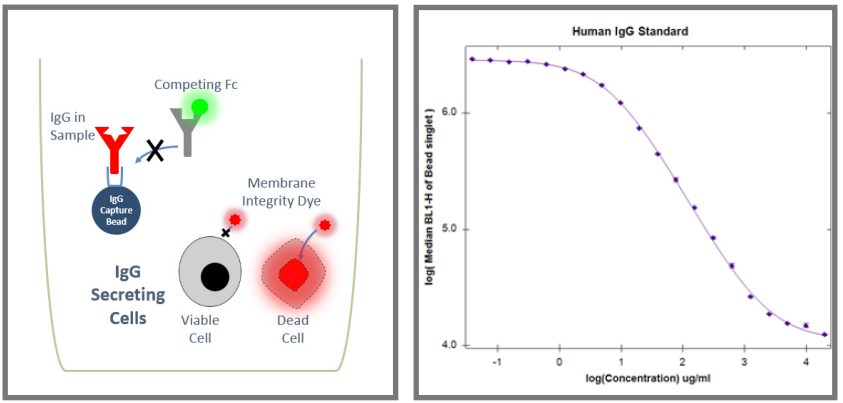


Figure 1. Principle of the Cy-Clone PLUS Kit. The no-wash competition assay functions on the differential binding of cell-secreted IgG vs recombinant FITC-IgG to IgG Capture Beads. Samples with high IgG concentrations will exhibit a low fluorescence signal, whereas the absence or low concentration of solution IgG would result in a large fluorescence signal.

Quantitative readouts from this assay can be measured as fluorescence intensity, or extrapolated to a concentration (µg/mL) in solution via the use of a standard curve. A standard curve from the assay shows a wide dynamic range. Two-fold dilutions of an IgG control standard were tested, and demonstrate a dynamic range of 0.6 µg/mL to 20 mg/mL.

Mix-and-Read Assay Workflow Overview

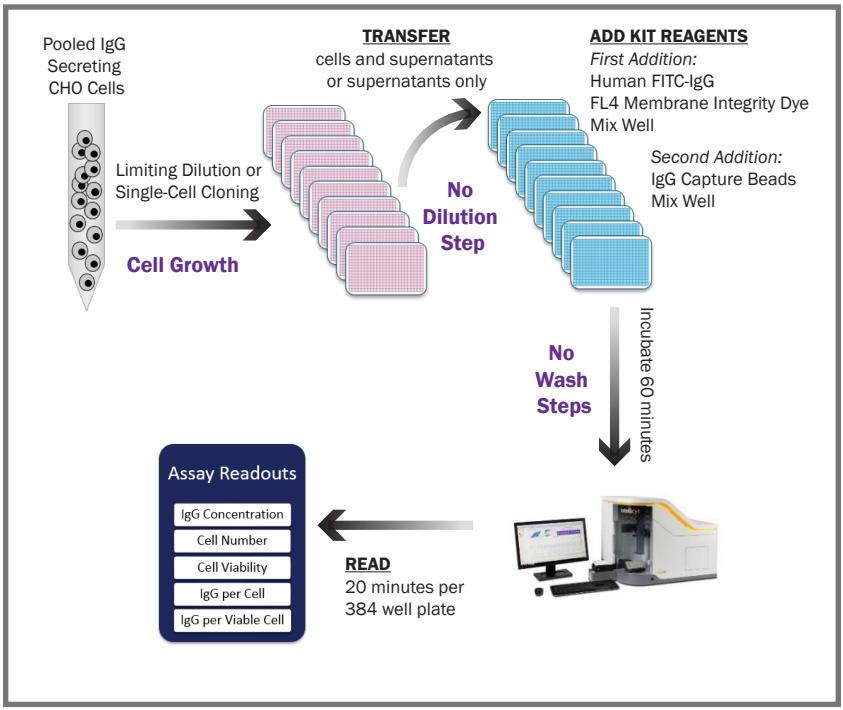


Figure 2. Screening Workflow. IgG secreting cells distributed into culture plates by standard methods including limiting dilution or single cell cloning are grown for the appropriate time. After growth, 20 µL samples from each well (including both cells and supernatants) are transferred to assay plates and mixed with FITC-IgG and cell viability dye first, and then mixed with IgG capture beads. After incubation at room temperature for 60 minutes, plates are read directly on the IntelliCyt Screening System.

Before Beginning

- Briefly centrifuge all vials before use to prevent reagent loss.
- Gently hand-mix or vortex the dye reagents prior to use
- Vigorously vortex beads prior to use to ensure homogenous solution and consistent concentration in assay. Beads tend to settle and aggregate over time
- Prepare working stock of reagents as follows:

Preparation of Sample Reaction Buffer

1. Add the entirety of the provided Sample Reaction Buffer solution to the lyophilized bovine serum albumin (BSA)
2. Mix gently until the BSA has completely dissolved. Excessive mixing or vortexing may cause foaming.

Preparation of Control Human IgG Protein Standard

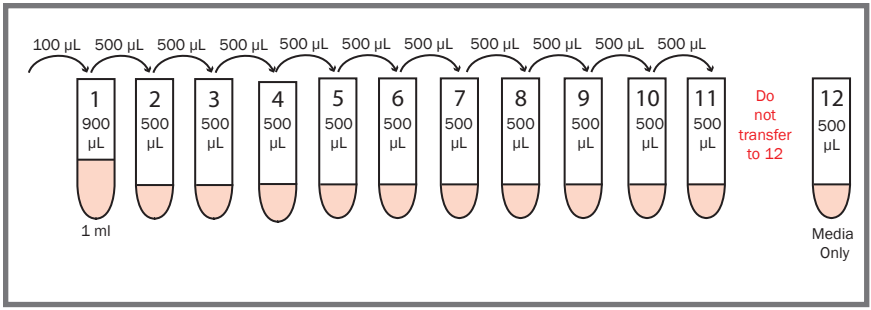
When preparing standards for quantitative protocols, using larger transfer volumes reduces the effects of pipetting error. We recommend that titration of standards is performed in at least 500 µL volumes, and that excess standard be discarded. The following protocol is a guideline that can be adapted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

1. Prepare 12 sample tubes (microcentrifuge tubes or 12x75 mm FACS tubes can be used), labeled #1 – 12.
2. To tubes # 2-12, add 500 µL of cell culture media and temporarily set aside.
3. To tube #1, add 1 mL of cell culture media. Add 20 µL of the provided Control Human IgG to the media and mix well. This sample is the highest concentration sample (200ug/mL) for the standard curve (top standard).

The Control Human IgG Protein Standard is provided as a solution at 10 mg/mL concentration. The instructions provided will yield a top concentration in the assay of 200 µg/mL, which will also constitute the highest point in the standard curve. If a higher top concentration is desired, the dilution factor and/or the volume of cell culture media used in each tube may be adjusted at Steps 2 and 3.

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 20 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.

Preparation of IgG Capture Beads

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

Preparation of Detection Reagents

	Sample Reaction Buffer	Human FITC-IgG (1:100 dilution)	FL4 Membrane Integrity Reagent (1:200 dilution)
1 x 384 wells	2.5 mL	25 µL	12.5 µL
5 x 384 wells	12.5 mL	125 µL	62.5 µL
20 x 384 wells	50 mL	500 µL	250 µL
50 x 384 wells	125 mL	1250 µL	625 µL

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

Protocol

This simplified protocol takes advantage of the no-wash work flow and allows for generation of results in terms of IgG concentration (e.g., µg/mL). This protocol involves preparing a serial titration of a reference protein to generate a standard curve which is used to determine IgG concentrations.

For ranking studies or assays where absolute concentrations are not required, the standard curve generation step may be omitted, and results can be reported as relative amounts of IgG in terms of median fluorescence intensity (MFI) which can be compared to controls.

Total Protocol Time: Approximately 1 hours 30 minutes

Total Hands-On Time: Approximately 15 minutes

Preparing Samples

1. Determine the approximate detection range for all samples to be tested. The Cy-Clone PLUS Kit detection range is 0.6 µg/mL – 20 mg/mL.
2. Obtain a blank (zero IgG) control that is prepared in the same culture media as the actual samples.

Perform the Assay

1. In each well of a 384-well plate, add 20 µL of sample or standards, and then add 5 µL of prepared detection reagent. Do a quick spin of the plate (500g, 5 seconds) to ensure that samples are at the bottom of the wells, not attached to the sides.

If performing assay in 96-well plates, the same protocol and protocol volumes may be used. IntelliCyt recommends the use of 96-well V-bottom plates. Shake speeds and other protocol settings may need to be adjusted to accommodate the 96-well plate format.

2. Mix the plate using a plate shaker and ensure thorough mixing. The shaker on the IntelliCyt screening system can be utilized for this step (20 seconds at 2,000 RPM).
3. Briefly vortex the prepared beads. Add 5 µL of prepared beads to each well. Agitate beads in the reservoir 1-2 times during the transfer of beads to a full assay plate to avoid beads precipitation to the reservoir bottom. After finishing the bead addition to the plate, Do a quick spin of the plate (500g, 5 seconds) to ensure that all samples are at the well bottom, not attached to the well sides.

Mix the IgG-containing sample or Control Human IgG with the Human FITC-IgG reagent **before** adding the IgG Capture Beads. This sequence of reagent addition is required for proper performance of the competition assay.

4. Mix the plate using a plate shaker and ensure thorough mixing. The shaker on the IntelliCyt screening system can be utilized for this step (20 seconds at 2000 RPM).
5. Incubate the plate, covered with a lid, at room temperature for 1 hour, protected from light.
6. Once the incubation is complete, directly acquire data on the IntelliCyt screening system. Refer to page 16. QSol buffer is recommended to be used in S1 cartridge (S1 rinse station) for the priming of the tubing.

Data Acquisition and Analysis

1. Launch ForeCyt Screening Software.
2. Create a new experiment using the pre-defined assay template provided with your kit. For detailed instructions on how to load and/or use IntelliCyt's assay templates, refer to the IntelliCyt 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.

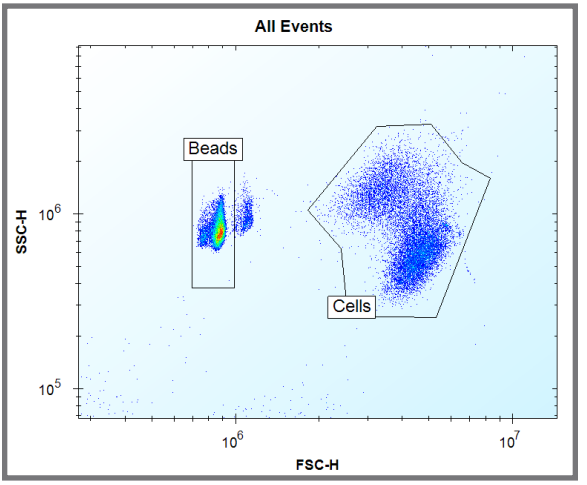
3. The default acquisition settings in bold are briefly explained below. If necessary, potential optimizations to each setting are shown in italics.

   - Pre-Plate Prime: **60 seconds**; Prior to sampling a plate, the system will prime the tubing with the S1 rinse station buffer (QSol buffer is recommended here) 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: **15 seconds at 2400 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.

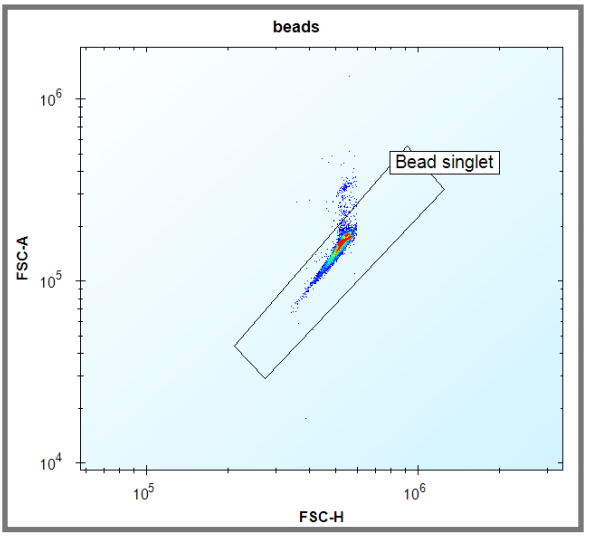
- Pump Speed: **Standard (15 rpm on iQue Standard, 29 rpm on iQue PLUS)**; Specifies the rate at which samples are introduced to the detectors. The default setting of standard roughly corresponds to introduction of ~1.5 µL per second. We recommend no adjustment.
- Interwell Shake: **4 seconds at 2400 RPM after every 12 wells if sampling in row direction or after every 16 wells if sampling in column direction.**; 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 events with size below 2 microns. We recommend no adjustment.

5. 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.

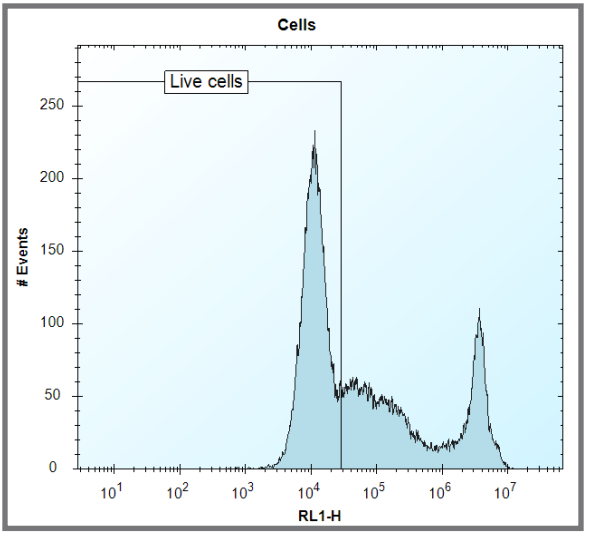
Step 1: Identify Cell and Bead Populations. If necessary, move the "cells" and "beads" gate to encompass the main region of interest as shown. If desired, the size of these gate can also be enlarged if additional cell populations are to be included in the analysis.



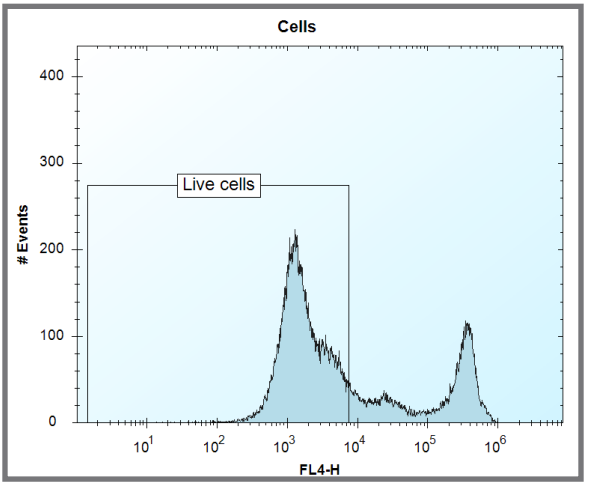
Step 2: Identify Singlet Beads. Analyzing only the single bead population helps avoid analysis artifacts created when aggregates of beads are analyzed. The singlet population will be seen on the ~45° angle on the FSC-H vs FSC-A plot.



Step 3: Identify Viable Cells. Binding of a DNA dye to the cells gives a measurement of cell viability in the FL4-H (iQue Screener standard) or RL1-H (iQue Screener PLUS) histogram. Positive cells (right peaks) represent the non-viable population. Adjust the gate as necessary to encompass the left-most peak, which represent the cells that are viable and have excluded the dye. This gate will be used to report the percentage of viable cells in each well.



Viability Gate on the iQue Screener PLUS

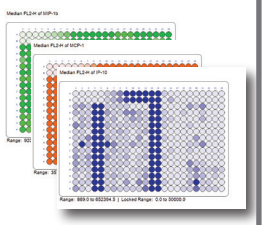


Viability Gate on the standard iQue Screener

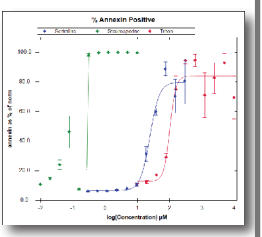
Visualization of Screening Results

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 ForeCyt Software features and instructional tutorials, please visit http://intellicyt.com/resources/welcome-forecyt-brief-orientation.

Heat Maps

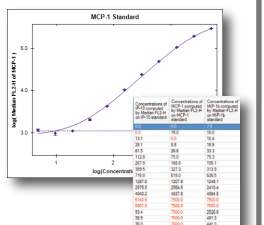


Dose Response Curves

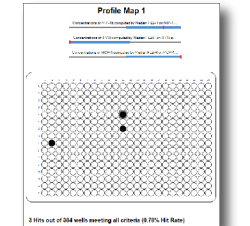


PDF Data Reports

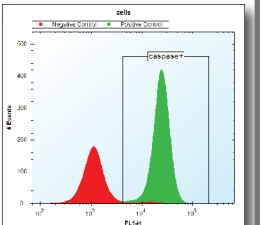
Standard Curves



Profile Maps



Overlay Plots



Appendix A: Mixing Samples with the IntelliCyt Shaker

iQue Screener and iQue Screener PLUS

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*

HTFC Screening System

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	3,000
384-Well	50+ µL	A/O*

iQue Screener HD

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	3,100
384-Well	50+ µL	A/O*
1536-Well	up to 5 µL	5,000

***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, IntelliCyt 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 IntelliCyt assay products.

Plate Type	Well Type	Manufacturer	Manufacturer Product #
384-well	V-bottom	Greiner	781280
96-well	V-bottom	IntelliCyt	10149

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	Aspiration Height Setting	Aspiration Height Offset	Aspiration 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

NOTES: _____

Abbreviated List of Consumables for IntelliCyt Screening Systems

iQue®/iQue® Screener PLUS and HTFC® Probes

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

iQue®/iQue® Screener PLUS and HTFC® Solutions

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

iQue®/iQue® Screener 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® (ForeCyt 4.0 or later req'd)
90636	FL2 Between-Well Marker Cartridge for iQue® (ForeCyt 4.0 or later req'd)
90637	FL3 Between-Well Marker Cartridge for iQue® (ForeCyt 4.0 or later req'd)
90638	FL4 Between-Well Marker Cartridge for iQue® (ForeCyt 4.0 or later req'd)

iQue®/iQue® Screener 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 Screener PLUS
91094	iQue® Screener PLUS Maintenance Kit
91095	iQue® Screener PLUS Maintenance Kit - 10 pack

**Refer to Intellicyt.com for complete list. Contact your local area sales representive for part number and pricing information

ABOUT THE COVER:

The Califonia Poppy (eschscholzia californica) also known as the golden poppy, California sunlight, or cup of gold, is a species of flowering plant native to the western and southwestern United States and Mexico. The plant grows approximately 5 to 20 inches tall, with teal colored leaves and bright golden flowers. The plant is used medicinally, culinarily, and cosmetically. As an herbal remedy, it has been used to treat insomnia, bedwetting (incontinence), anxiety and nervous tension, as a memory aid, analgesic, and as an antimicrobial tincture.

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