SARTORIUS

Product Guide

# iQue® In-Well Markers

## Product Information

iQue® In-Well Markers are fluorescent beads designed for facilitating well identification in iQue Forecyt®, and can be used on iQue® HTFC, iQue® and iQue® PLUS platforms. Each kit provides sufficient beads run up to 10 x 384 well plates. The In-Well Markers can be used for any assay ran in 96-, 384-, or 1536-plates although volumes may have to be adjusted to accommodate the different plate densities. Prepared marker beads are added directly to each well of the assay, immediately before data acquisition. The kit facilitates the automated identification of individual wells, and is especially useful in assays where well-towell cell counts vary widely, or where cell numbers per well are low (ie. toxicity assays, clone selection assays).

The starter kits are provided in configurations of either 4 colors (for iQue® HTFC or iQue® standard) or 5 colors (for iQue® Screener PLUS) of marker beads, and is designed for flexibility to in selecting the appropriate bead during assay development. Single color kits are meant for larger-scale experiments that have been optimized to work with a specific marker bead type. Appropriate labeling buffers for all bead colors are also provided. The following protocol details the procedure to properly prepare the beads, and their use | addition to an assay plate.

### Kit Contents

- Intra-Well Marker beads (either of a single bead color, or one of each color for the starter pack)
- Sample Reaction Buffer (liquid)
- Component A (lyophilized)

Note: Buffers are only supplied with 10 x 384-well plate sizes. For larger kit sizes, a user specified buffer should be utilized. Standard laboratory buffers such as Hank's Balanced Salt Solution (HBSS), Phosphate Buffered Saline (PBS) or tissue culture media , with protein (bovine serum albumin, or serum) ranging from 0.1 – 1% can be used.

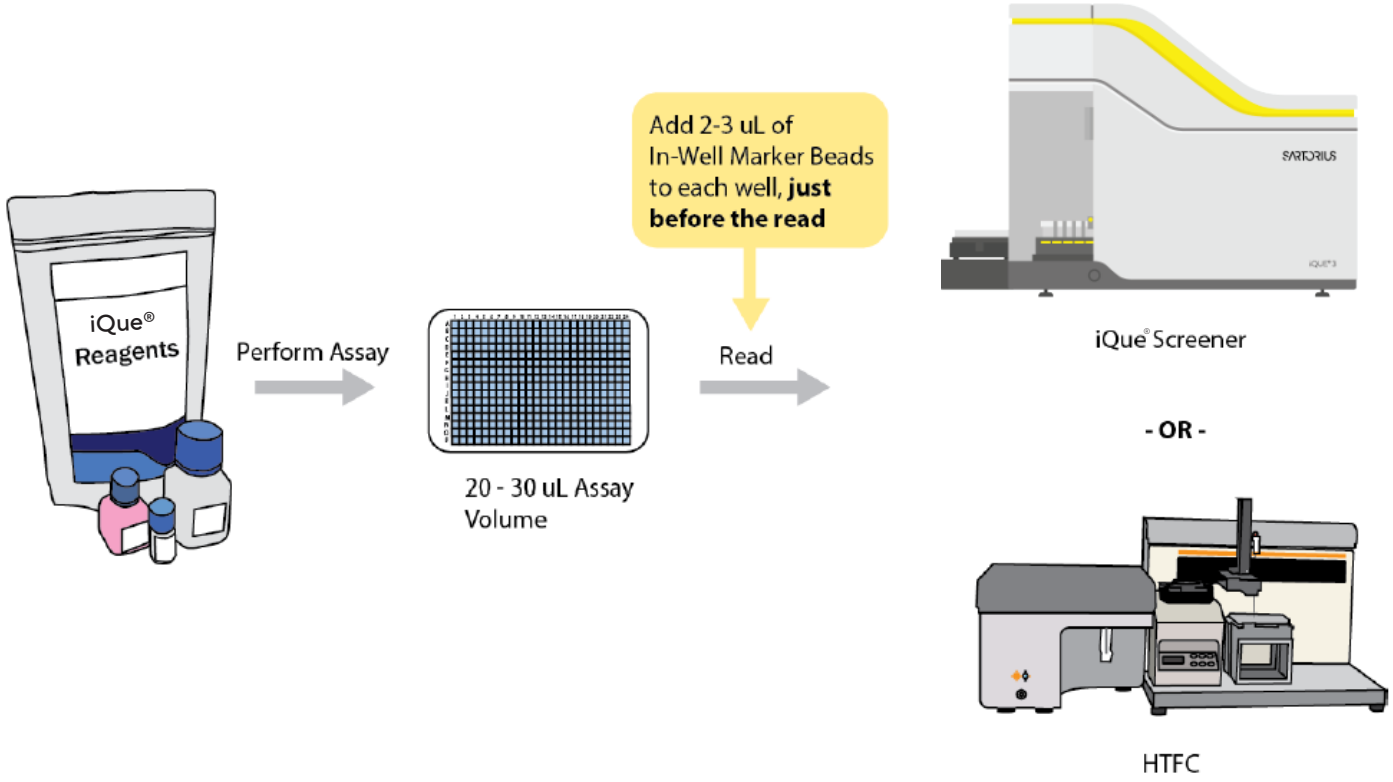
### Storage

Store at 2–8°C. Protect from prolonged exposure to light. Do not freeze.

### Materials Needed but Not Provided

- Assay Plates
- 96-well V-bottom plates (iQue® #90151)
- 384-well V-bottom plate (Greiner® #781280)
- iQue® platform equipped with iQue Forecyt®

# Workflow Overview




# Before You Begin

Prepare working stock of beads in provided dilution buffer

| Kit Size | Kit Volume (mL) | Dilution Factor | Buffer Volume (mL) to Add | Total Prepared Volume (mL) |
|---|---|---|---|---|
| 10 x 384-well | 2 | 1:5 | 8 | 10 |
| 100 x 384-well | 20 | | 80 | 100 |
| 250 x 384-well | 50 | | 200 | 250 |
| 500 x 384-well | 100 | | 400 | 500 |
| 1000 x 384-well | 200 | | 800 | 1000 |

**Table 1:** Working stock of beads

Note: Buffers are only supplied with 10 x 384-well plate sizes. For larger kit sizes, a user specified buffer should be utilized. Standard laboratory buffers such as Hank's Balanced Salt Solution (HBSS), Phosphate Buffered Saline (PBS) or tissue culture media , with protein (bovine serum albumin, or serum) ranging from 0.1 – 1% can be used.

The volumes above are specified to utilize a full kit at a time. To prepare marker beads for fewer plates, dilute beads at the indicated dilution factor in an appropriate buffer.

# Procedure

**1. Selecting the Appropriate Bead Color for your Experiment**

Each marker bead is designed for optimal detection on the specified fluorescence channel, although the fluorescence signal can overlap and be detected on other channels. It is recommended that a marker on an unoccupied fluorescence channel be selected for use. For example, if an experiment contains a GPF reporter B/Green (FL1), use of the B/Green In-Well Marker is not recommended. Select B/Yellow (FL2), B/Red (FL3), or R/Red (FL4) for optimal performance. For experiments where all channels are occupied for biological endpoints, the channel with the dimmest assay signal could potentially be utilized for in-well beads, but this will need to be established for each specific assay. For use with iQue® Human 4-Plex Apoptosis Kit, B/Yellow or B/Red In-Well Markers can both be utilized.

**2.0 Perform Your Assay**

With the use of the In-Well Markers, there is no need for modification of your assay protocols. Perform your assay as normal.

**3.0 Adding Marker Beads to Samples**

Once the assay is complete and ready for data acquisition, add prepared marker beads to each well. The beads should be added to each well at a 1:10 fold dilution to achieve optimal density for sampling.

For instance:

- if the final well volume is 20 µL, add 2 µL per well of prepared beads.
- if the final well volume is 30 µL, add 3 µL per well of prepared beads.

Mix samples after addition to ensure that beads are homogenously dispersed before sampling. The shaker on your iQue® platform can be used for this step.

**4.0 Acquiring Data with In-Well Markers**

With the use of the In-Well Markers, there is no need for modification of your data acquisition protocols. Acquire data from your assay using a standard protocol.

**5.0 Data Analysis: Performing Well ID based on In-Well Beads**

Once data acquisition is complete, click on the Well Identification tab in iQue Forecyt®.

5.1 Under the Well Identification tab, click and open the noise gate icon. 

5.2 Change the axes to the FL parameter corresponding to the marker bead color used

<u>iQue® Standard: 4 Color Options</u>

i. B/Green (FL1): x-axis FL1-H, y-axis FL4-H
ii. B/Yellow (FL2): x-axis FL2-H, y-axis FL4-H
iii. B/Red (FL3): x-axis FL3-H, y-axis FL2-H
iv. R/Red (FL4): x-axis FL4-H, y-axis FL2-H

<u>iQue® PLUS: 5 Color Options</u>

i. B/Green (BL1): x-axis BL1-H, y-axis RL1-H
ii. B/Yellow (BL2): x-axis BL2-H, y-axis RL1-H
iii. B/Red (FL4): x-axis BL4-H, y-axis BL2-H
iv. R/Red (RL1): x-axis RL1-H, y-axis BL2-H
v. V/Blue (VL1): x-axis VL1-H, y-axis SSC-H

5.3 Draw a noise gate around the bead population. Typically, the beads will form a small cluster at the far right of the x-scale (**Figure 2**).

5.4 Select Calculate Wells to perform Well ID.

5.5 Once wells have been identified with marker beads, downstream data analysis steps are not affected by the presence of beads.

5.6 Click on the Analysis tab to continue analyzing assay data.

5.7 Expected bead counts, per well, based on sip time

i. 1 second ~ 150 – 200 beads
ii. 3 second ~500 – 600 beads

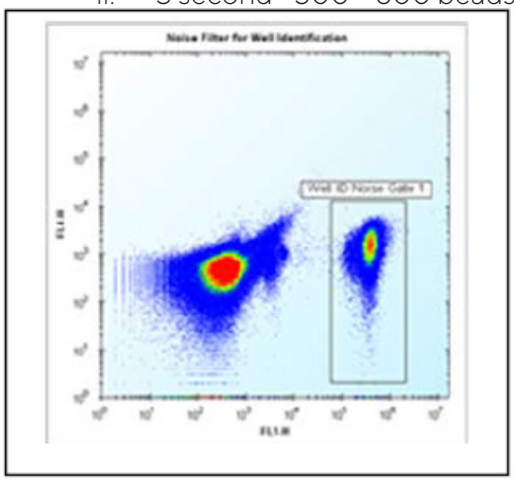


**Figure 2.** Setting of Noise Gate on FL-1 In-well Marker Beads. Beads are typically brightest fluorescence (far right) signal.

| Problem | Possible Cause | Recommended Action |
|---|---|---|
| Collected bead numbers are low | Beads not properly diluted<br><br>Assay plate well volumes too high and \| or not enough beads added to wells | Consult protocol for appropriate bead dilution<br><br>Ensure enough volume of beads have been added to all sample wells |
| Not all wells have been identified | Sampling issues prevented beads from being sampled appropriately<br><br>Not enough beads per well have been acquired | Identify and correct any sampling errors that may have been encountered. This may include:<br>▪ **Incorrect instrument calibration**<br>▪ **Clogged sample line \| cytometer**<br>▪ **Incorrect acquisition threshold(s)**<br>Increase sip time to acquire more beads |
| Cannot find bead population | Noise filter axis parameters not correct<br><br>Incorrect bead used: bead fluorescence overlapping with assay fluorescence | Ensure that the proper parameters are selected for the x- and y-axis, for the proper bead fluorescence<br><br>Consult Marker Bead Wizard to ensure appropriate bead used |
| Not enough bead volume for plate | Beads not properly diluted | Consult protocol for appropriate bead dilution<br><br>Ensure enough volume of beads have been added to all sample wells |

**Table 2.** Troubleshooting Guide

# Sales and Service Contacts

## For further contacts, visit www.sartorius.com

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