SARTORIUS

Technical Note

Intracellular Staining Assay for iQue® Platform

For the quantification of intracellular markers

This technical note provides an overview of the method for staining intracellular markers in adherent and suspension cell lines for analysis on the iQue® Advanced Flow Cytometry Platform. It combines the iQue® Fixable Viability Kits with a variety of conjugated antibodies targeting intracellular markers specific to the cells of choice. For further information regarding the iQue® Fixable Viability Kits, please refer to the product guides.

Required materials

- iQue® Fixable Viability Dye kit Sartorius, Cat. No. BA-97116
- Phosphate buffered saline (PBS)
- MPO Fixation buffer (BioLegend; Cat. No. 78078)
- Fixation buffer (BioLegend; Cat. No. 420801)
- Intracellular Permeabilization Wash buffer (BioLegend; Cat. No. 421002)
- Cells of interest
- Cell culture media/culture conditions
  96-well v-bottomed microplate (e.g. Corning®, Cat. No. 3363 3363)
- Conjugated antibodies with appropriate controls
- Centrifuge with plate adaptors

The protocol described is a method for staining intracellular markers in a variety of cell types, both adherent and non-adherent.

Quick Guide

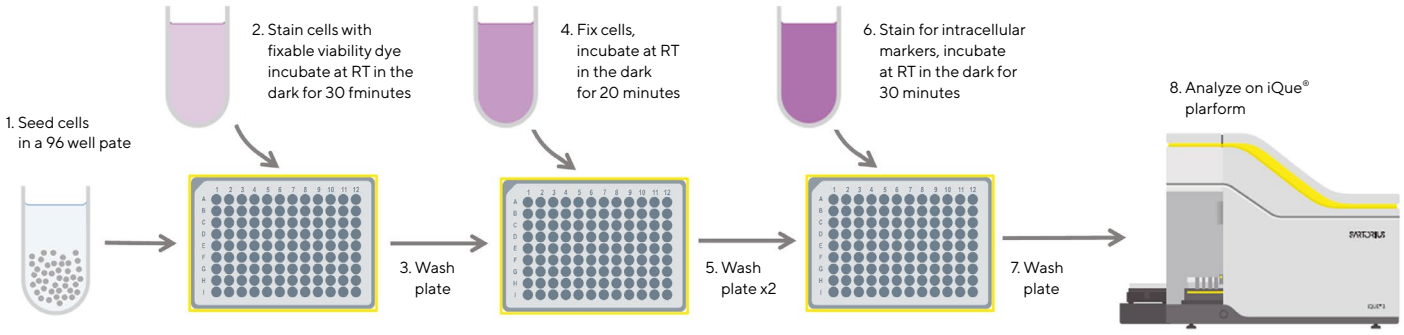


Figure 1 Workflow for intracellular staining. Surface marker staining can be performed during this incubation step at the same time the cells are stained with the fixable viability dye.

Protocol

iQue Fixable Viability Dye preparation

1. Allow iQue® Fixable Viability Dye and DMSO to reach room temperature prior to assay.
2. Reconstitute one vial of lyophilized dye by adding 100 µL DMSO to the dye vial and mix until fully dissolved. This is the 200X dye stock solution.
3. Prepare a 1X Dye Staining Solution by adding 10 µL of the 200X dye stock solution per 2 mL PBS.

Viability Staining

1. Harvest and dissociate your chosen cell lines and plate 2 × 104 cells in 10 µL of medium per well in a V-bottom 96-well plate.
2. Wash cells once by adding 90 µL of PBS and centrifuging at 400 x g for 5 minutes, aspirate supernatant.
3. Shake plate at 3000 RPM for 60 seconds.
4. Add 10 µL of the prepared 1X Dye Staining Solution to each well.
5. Centrifuge the assay plate at 400 x g for 5 seconds and shake briefly at 2000 RPM for 20 seconds.
6. Incubate at RT in the dark for 30 mins.
7. Add 90 µL PBS +0.5% BSA to the wells and centrifuge for 5 mins at 400 x g.
8. Aspirate supernatant and shake plate at 3000 RPM for 60 seconds.

Fixation and Permeabilization

1. Add 10 µL of Fixation buffer to each well.
2. Spin the assay plate briefly at 400 x g for 5 seconds and then shake for 20 seconds at 2000 RPM.
3. Incubate samples at RT in the dark for 20 minutes.
4. Add 90 µL Permeabilization Wash buffer to each well. Centrifuge at 400 x g for 5 mins. Aspirate supernatant.
5. Add 100 µL Permeabilization Wash buffer to each well. Centrifuge at 400 x g for 5 mins. Aspirate supernatant.
6. Shake the plate at 3000 RPM for 60 seconds.

Intracellular staining

1. Add 10 µL of the antibodies of choice diluted in Permeabilization Wash buffer to each well.
2. Briefly centrifuge the plate at 400 x g for 5 seconds and then shake at 2000 RPM for 20 seconds.
3. Incubate the samples at RT in the dark for 30 mins.
4. Add 90 µL permeabilization/wash buffer per well.
5. Centrifuge at 400 x g for 5 mins, aspirate supernatant and shake at 3000 RPM for 60 seconds.
6. Add 20 µL PBS + 0.5% BSA to each well and run on the iQue® platform.

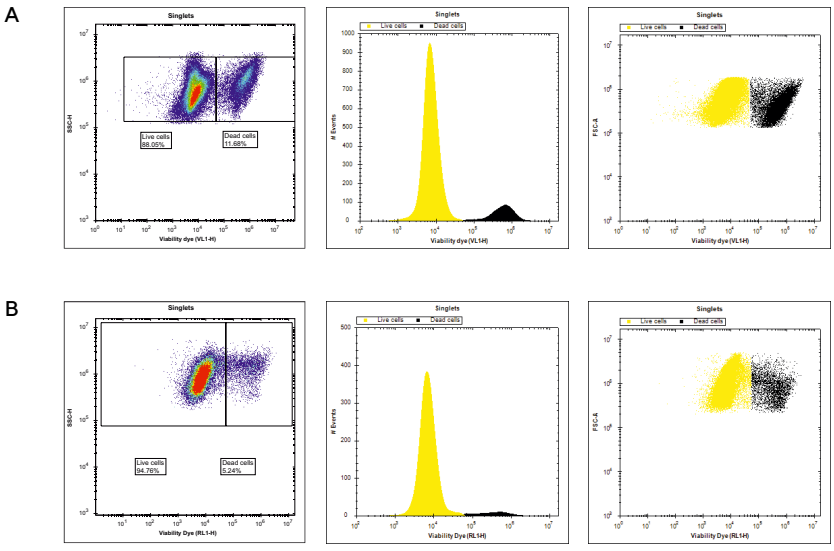
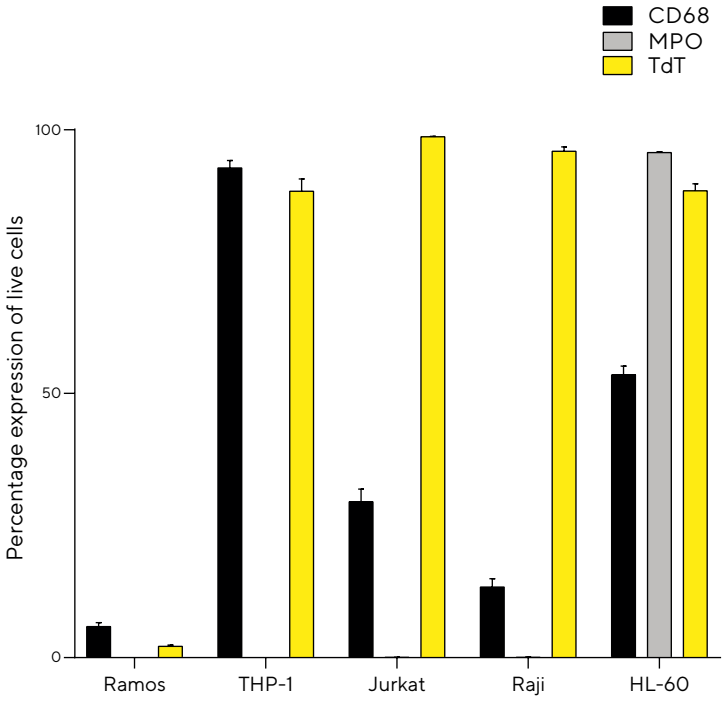


Figure 2. Fixable viability dyes enable reliable live/dead analysis in fixed samples. Suspension (A) and adherent (B) cells were stained with the fixable viability dyes described in this protocol and gated for live and dead populations. The figure displays a standard dot plot of live/dead cells, a histogram of the same data and a further dot plot highlighting the live and dead populations in fixed colours.



Marker	Expressed in
CD68	Granules of Macrophages
MPO	Myeloid cells/Neutrophil granulocytes
TdT	T cells, thymocytes, subset of B lymphoblasts

Cell type	Characteristics
Ramos	B Lymphocyte – Burkitt's Lymphoma
THP-1	Monocyte – acute monocytic leukaemia
Jurkat	T Lymphoblast – acute T cell leukaemia
Raji	B Lymphocyte – Burkitt's Lymphoma
HL-60	Promyeloblast - acute promyelocytic leukaemia

Figure 3. Intracellular staining of several immunologically relevant cell lines showcases the power and flexibility of the iQue® platform. Various suspension cell lines were stained with 3 intracellular markers specific to different lineages of haematopoietic cells. Each cell line was stained with all three antibodies in the same well of a 96 well plate, providing a high throughput solution to staining for multiple markers.

Analysis

1. Once your plate has run, you can select different methods for displaying your data, and manipulate gates and create new plots and histograms.
2. Heatmaps are also an option for a visually clear method of displaying data.

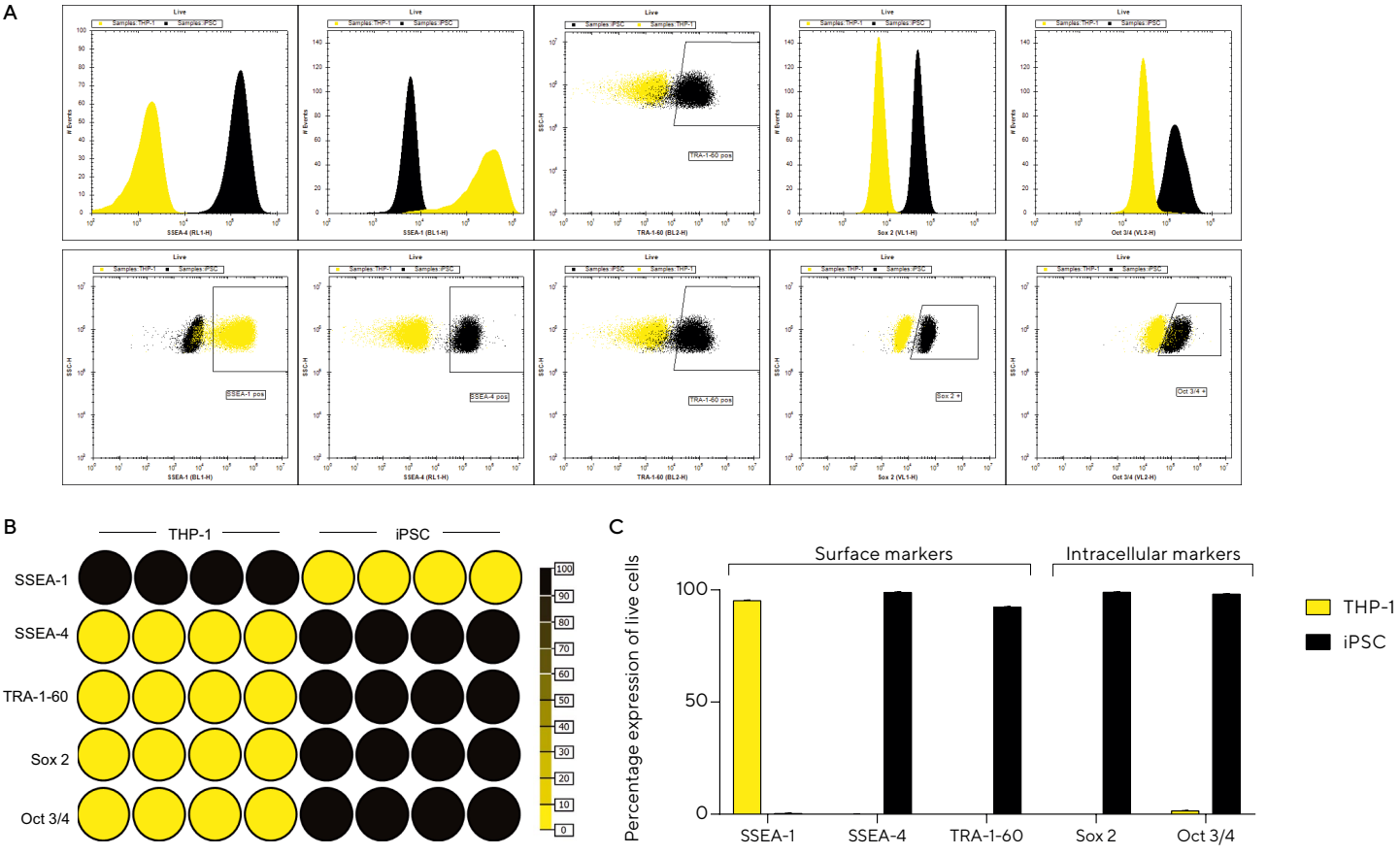


Figure 4. Surface and intracellular marker staining provides solutions for high throughput cellular characterization. Stem cell pluripotency can be analyzed using the intracellular staining protocol outlined here in combination with surface marker characterization in the same well. SSEA-1 was used as a marker of normal, non-pluripotent cells, while SSEA-4, TRA-1-60, Sox 2, and Oct 3/4 were all used to characterize pluripotent cells. Histograms and dot plots created in the Forecyt software system for iQue, showing the expression of various surface and intracellular markers in iPSC and control cells (A). Heatmap from iQue Forecyt® illustrating the expression of the same markers, representing the plate map and expression profile per well (B). Bar graph expressing marker data in 3rd party software (C).

Ordering Information

Platform: Compatible with iQue® 3/ iQue® Screener Plus – VBR and BR Configuration

Product	Size	Catalog Number
iQue® Fixable Viability (V/Blue) Kit	5 × 96 Wells	BA-97116
iQue® Fixable Viability (B/Green) Kit	5 × 96 Wells	BA-97119
iQue® Fixable Viability (R/Red) Kit	5 × 96 Wells	BA-97122

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