Mouse kidney cell staining protocol for FACS sorting

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Jun 23, 2021

Human Cell Atlas Method Development Community

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ABSTRACT

A brief description on staining and gating for FACS sorting the live cells from dissociated mouse kidney tissue

DOCUMENT CITATION

Xian Adiconis 2021. Mouse kidney cell staining protocol for FACS sorting. **protocols.io** https://protocols.io/view/mouse-kidney-cell-staining-protocol-for-facs-sorti-bv2yn8fw

CREATED

Jun 23, 2021

LAST MODIFIED

Jun 23, 2021

DOCUMENT INTEGER ID

51000

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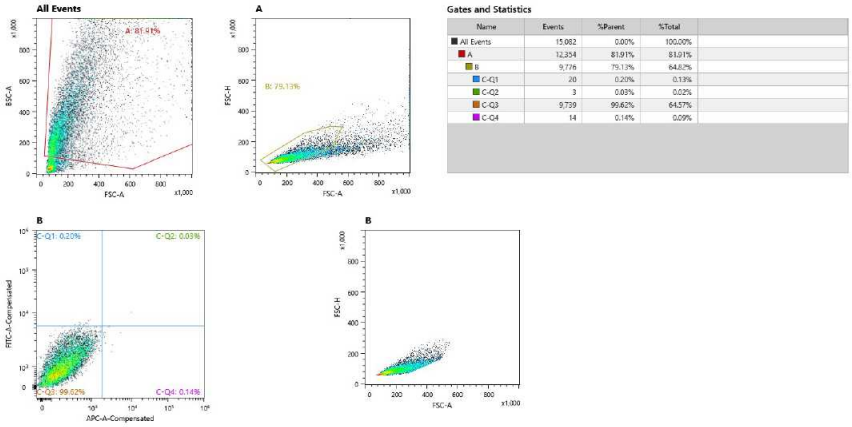
Reagents:

1. TO-PRO™-3 Iodide (642/661)- 1 mM Solution in DMSO (ThermoFisher Scientific, T3605)
2. Calcein, AM (ThermoFisher Scientific, C3099)

Due to the significant presence of debris (TO-PRO3 negative as the live cells), both dead cell (TO-PRO3) and live cell (Calcein) stains are needed to detect the live cells. Due to the high auto fluorescent signal of the dissociated tissue, it is essential to set aside a small amount unstained cells as the negative control for gate setting.

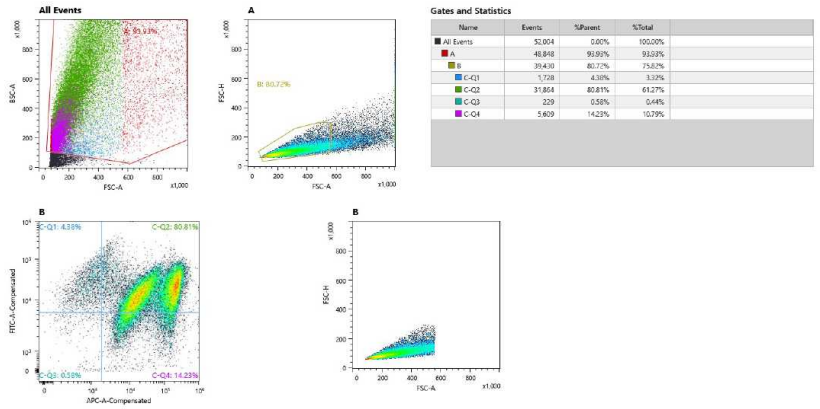
Make 1/1000x dilutions of above dead and live cell stain, add 3-5µl each of the diluted stain to each ml of the cell suspension 15 minutes prior to FACS sorting.

Based on the negative control signal, gate on TO-PRO3 (dead cell stain) negative and Calcein (live cell stain) positive (area C-Q1) for the stained sample for live cell sorting.



Name	Events	%Parent	%Total
All Events	15,062	0.00%	100.00%
A	12,354	81.91%	81.91%
B	9,736	79.13%	64.65%
C-Q1	20	0.20%	0.13%
C-Q2	3	0.03%	0.02%
C-Q3	9,739	99.62%	64.57%
C-Q4	14	0.14%	0.09%

Unstained cell plots, from SONY (SH800)



Name	Events	%Parent	%Total
All Events	52,004	0.00%	100.00%
A	48,848	93.93%	93.93%
B	39,430	80.72%	75.82%
C-Q1	1,728	4.38%	3.32%
C-Q2	31,864	80.81%	61.27%
C-Q3	229	0.58%	0.44%
C-Q4	5,609	14.23%	10.79%

Stained cell plots, from SONY (SH800)