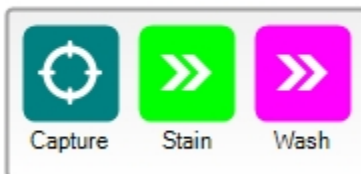


Name Cell Stain by Tra-1-60
Revision B
Description Cell Load & Stain Script for use with Fluorescent (Directly-Conjugated) Antibodies against Cell Surface Epitopes
Authors Cell Biology
Institution Fluidigm Corporation
Lab Research & Development
Special Instructions

Cell Load and Stain



Script Summary - Prime

Runtime Estimates

Barcode	Estimate
1861x (5-10 um diameter cells)	0 hours, 11 minutes
1862x (10-17 um diameter cells)	0 hours, 13 minutes
1863x (17-25 um diameter cells)	0 hours, 12 minutes

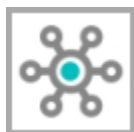
Script Summary - Cell Load and Stain

Runtime Estimates

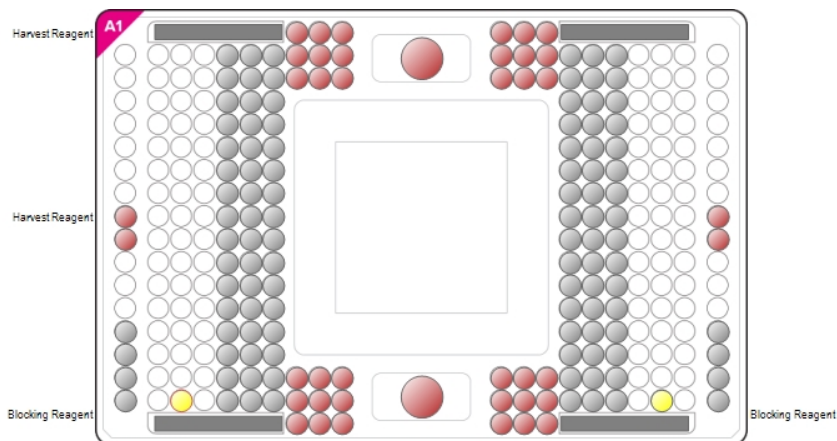
Barcode	Estimate
1861x (5-10 um diameter cells)	0 hours, 31 minutes
1862x (10-17 um diameter cells)	1 hours, 0 minutes
1863x (17-25 um diameter cells)	0 hours, 51 minutes

Incubation Profile

Script Step	Operation	Temperature (C)	Duration (s)
Stain	Incubation	25	600



Script Reagent Details - Prime



Reagent Loading

Name	Volume (μl)	IFC Inlet	Notes
● Harvest Reagent	200 μl	A1	
● Harvest Reagent	200 μl	A2	
● Blocking Reagent	15 μl	C1	
● Blocking Reagent	15 μl	C2	
● Harvest Reagent	20 μl	P1	
● Harvest Reagent	20 μl	P2	

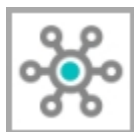
Reagent Mix Recipe - Prime

Blocking Reagent

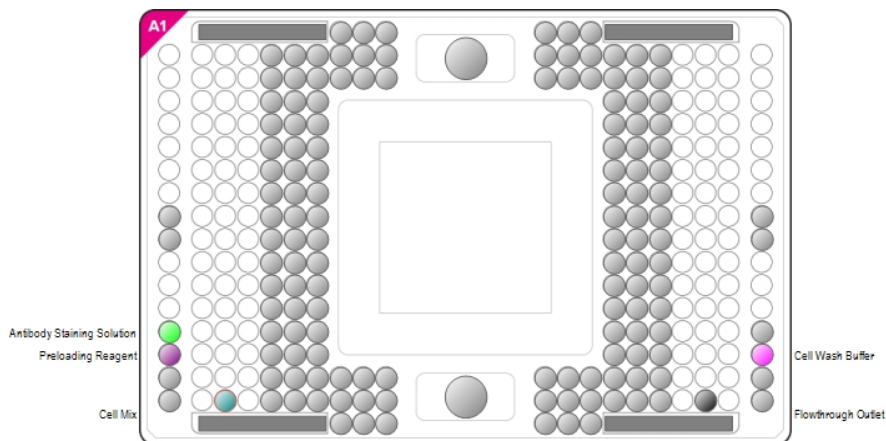
Reagent (Stock Concentration)	Mix Prep (μl)	Prep Conc.	Chamber Conc.
C1 Blocking RGT (1X)			

Harvest Reagent

Reagent (Stock Concentration)	Mix Prep (μl)	Prep Conc.	Chamber Conc.
C1 Harvest RGT (1X)			



Script Reagent Details - Cell Load and Stain



Inlet Reuse

Name	IFC Inlet	Instructions
● Cell Mix	C1	Aspirate inlet prior to loading reagents
● Flowthrough Outlet	C2	Aspirate inlet prior to loading reagents (1862x, 1863x only)

Reagent Loading

Name	Volume (μl)	IFC Inlet	Notes
● Antibody Staining Solution	7	1	
● Preloading Reagent	20	2	
● Cell Wash Buffer	7	6	
● Cell Mix	6	C1	

Reagent Mix Recipe - Cell Load and Stain

Preloading Reagent

Reagent (Stock Concentration)	Mix Prep (μl)	Prep Conc.	Chamber Conc.
C1 Preloading RGT (1X)			

Cell Mix

Reagent (Stock Concentration)	Mix Prep (μl)	Prep Conc.	Chamber Conc.
C1 Suspension RGT (2.5X)	40	1	1
Cells 66 - 330 / μL	60		

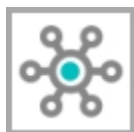
100 Total Prep Volume

Antibody Staining Solution

Special Instructions:

Prepare a stock of your conjugated-antibody at 2x the manufacturer's concentration in Ce Cell Wash Buffer. For this example, the manufacturer calls for 5ug/ml in the recipe, use 10ug/ml instead as a final concentration.

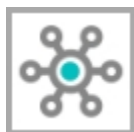
You may also include a counter stain of CellTracker Orange CMRA (Life Technologies) at a final concentration of 5 micromolar in this stock to stain all cells.



Reagent (Stock Concentration)	Mix Prep (μl)	Prep Conc.	Chamber Conc.
C1 Cell Wash BUF (1X)	46.5	0.93	0.93
Antibody (0.5 mg/ml)	1	0.01	0.01
CellTracker Orange (0.1 mM)	2.5	0.005	0.005

50 Total Prep Volume

Cell Wash Buffer			
Reagent (Stock Concentration)	Mix Prep (μl)	Prep Conc.	Chamber Conc.
C1 Cell Wash BUF (1X)			

**Protocol Reagent Shopping List**

Reagent Name	Vendor	Part Number	Kit Part Number	Stock Concentration
Antibody	Stemgent	09-0068		0.5 mg/ml
CellTracker Orange	Life Technologies	C34551		0.1 mM

Fluidigm Reagent Kits

Reagent Name	Part Number	Stock Concentration	PN 100-8920	PN 100-6201	PN 100-5319	PN 100-7357	PN 100-8921
C1 Blocking RGT	100-5316	1X	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	
C1 Harvest RGT	100-6248	1X	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>			<input checked="" type="checkbox"/>
C1 Preloading RGT	100-5311	1X	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	
C1 Suspension RGT	100-5315	2.5X	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	
C1 Cell Wash BUF	100-5314	1X	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>		