

Pala Cell Sorter & Single Cell Dispenser

Quick Reference Guide

PALA 488/561 AND PALA 405/488

Available Fluorophores and Channels

Filter	488 nm	561 nm
527/32	FITC, GFP	
586/42	Nile Red	PE
625/15	488/625	mCherry
692/80	PerCP, PI	PE-Cy5
783/56	488/783	PE-Cy7

Filter	405 nm	488 nm
447/45	BFP	
527/32	BV510	FITC, GFP
586/42	BV750	PE/DsRed
625/15	BV605	PE-Texas Red
692/80	BV650	PerCP, PI
783/56	BV785	PE-Cy7

Single Cell Mode

- Compatible with 96 or 384 well plates.
- Cell concentration:
~10,000-20,000 cells/ml
- Use cell culture grade water for sheath fluid.
- Keep events at 1-2 events/sec to ensure accurate single-cell deposition.
- Target population % will dictate speed.
At 80% target population, expect 96-well plate to take ~2 mins.












Bulk Mode

- Ideal for 5,000 – 50,000 target cells dispensed into a 1.5 or 2 ml tube.
- Cell Concentration:
~100,000-500,000 cells/ml
- Use PBS or media (without FBS) for sheath fluid.
- Keep events under 50 events/sec to maximize purity.
- A mixed population of cells that contain 20% of target cells will take ~10 minutes to sort/enrich 5,000 cells.


Before Getting Started


- Login for the laptop is "Pala" and the Password is "Pala".
- Filter cells with ~40 µM filter to avoid clogging.
- Run the cleaning protocol to sterilize the Pala.
- Prepare plates with outgrowth media and store in incubator.
- Use a hemocytometer or automated cell counter to ensure accurate cell count.
- Have cells ready at appropriate concentration prior to dispensing.
- Use a viability stain such as Cell Tracker Red or Green for best results.







Software Icons


- | | | |
|--|---|---|
|  Initialize System |  Cancel Action |  Switch Cartridge |
|  Shut Down System |  Analyze Sample |  Cleaning Protocol |
|  Single Cell Dispense |  Bulk Cell Sorting |  Settings Control Menu |

Steps for Single Cell Dispensing into a plate


1. Fill the Sheath bottle with purified, filtered water and empty the waste bottle.
2. Initialize  the Pala and follow the screen prompts (~5 minutes).
3. Pipette 100–600 µl of cells into a Single-Cell Cartridge (PN NC0003). Cells should be at a concentration of 10,000–20,000 cells per ml.
4. When a prompt appears, select cartridge type. Insert the cartridge and ensure the cartridge is properly oriented. The cartridge holder will automatically open and close after the series of prompts.
5. Adjust the silver positioner so that the left dark line is adjacent to the left light line in the camera image.


Warning:  Improper alignment of the cartridge can cause a decrease or loss in events displayed.







6. Select the channels you would like to capture on Charts 2–6 on the bottom of the screen (example FITC x PE) as well as the chart type (Scatter, Histogram, Density Plot). FSC vs TSC is the default for Chart 1.
7. Select  to start analysis of your sample. Right-click on any of the charts to create a gate (polygonal, quadrant, or histogram). To move a polygonal gate, hold down “ctrl” on the keyboard, click on the gate with the mouse depressed and move to desired position.
8. Gain adjustments to the scatter and fluorescent detectors can be accessed through the  Settings Control Menu. After making adjustments, click on to accept the changes.
9. The optimal event rate is between 1–2 events per second. The sample rate can be increased or decreased if the event rate is above this range.
10. Select  to stop analysis and select the plate type and wells.
11. Select plate type and wells and place the microtiter plate prefilled with media onto the plate stage. Select  to start dispensing. Default is 1 cell per well, but this can be changed in the plate map.
12. Perform a cartridge switch  to replace the cell cartridge with the cleaning cartridge to process the next sample or to add additional sample to the same cartridge.
13. Shutdown  the system and follow the software prompts.


Warning:  Failure to shutdown the system with anything but water in the Sheath Bottle can cause permanent damage to the fluidic lines.

Steps for Bulk Cell Dispensing into a tube

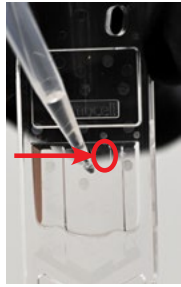
1. Fill the Sheath bottle with PBS or media (no FBS) and empty the waste bottle. Water can not be used as sheath fluid as it will lyse the cells after sorting.
2. Initialize  the Pala and follow the screen prompts (~5 minutes).
3. Pipette 100–600 µl of cells into a Bulk-Cell Cartridge (PN NC101). Cell concentration should be between 100,000 to 500,000 cells/ml.
4. When a prompt appears, select cartridge type. Insert the cartridge and ensure the cartridge is properly oriented. The cartridge holder will automatically open and close after the series of prompts.
5. Adjust the silver positioner so that the left dark line is adjacent to the left light line in the camera image.

Warning:  Improper alignment of the cartridge can cause a decrease or loss in events displayed.

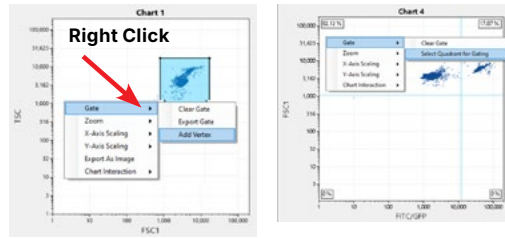
6. Select the channels you would like to capture on Charts 2–6 on the bottom of the screen (example FITC x PE) as well as the chart type (Scatter, Histogram, Density Plot). FSC vs TSC is the default for Chart 1.
7. Select  to start analysis of your sample. Right-click on any of the charts to create a gate (polygonal, quadrant, or histogram). To move a polygonal gate, hold down “ctrl” on the keyboard, click on the gate with the mouse depressed and move to desired position.
8. Gain adjustments to the detectors can be accessed through the  Settings Control Menu. After making adjustments to the detectors, to accept the changes.
9. The ideal event rate should be 50 events per second or less. The sample rate can be increased or decreased if the event rate outside this range.
10. Select  to stop analysis.
11. Enter the desired number of cells to be sorted.
12. Select  to initiate the bulk dispensing steps. Ensure that the microcentrifuge tube insert (black metal ring) is seated into the plate stage and place a microcentrifuge tube into the insert.
13. Perform a cartridge switch  to replace the cell cartridge with the cleaning cartridge to process the next sample or to add additional sample to the same cartridge.
14. Shutdown  the system and follow the software prompts.

Warning:  Failure to shutdown the system with anything but water in the Sheath Bottle can cause permanent damage to the fluidic lines.

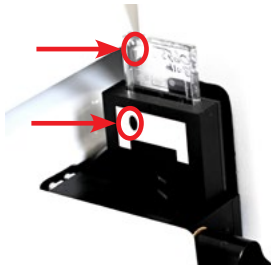
Step 3



Step 7



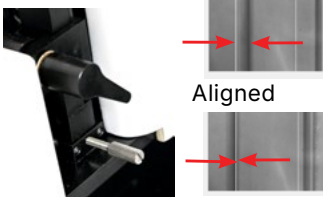
Step 4



Step 8

Amplifier	Gain	Laser(s)	Emission Filter	Fluorophore(s)
FSC	900	488/405	488-10	FSC1
TSC	800	488	488-10	TSC
PM11	250	405	447-45	BFP
PM12	250	488/405	527-32	FITC/BV510
PM13	500	488/405	568-42	PE/BV70
PM14	300	488/405	625-15	PE-Texas Red/BV605
PM15	500	488/405	682-80	PerCP/BV650
PM16	800	488/405	783-56	PE-Cy7/BV785

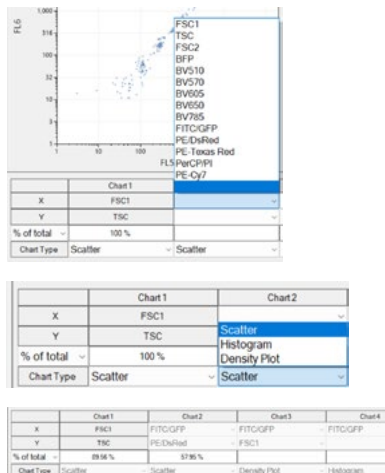
Step 5



Step 9

Sample Rate: 5
 Acquisition Number: 1
 Plate Format: 96
 Select All
Event Rate: 1.9 events/sec (avg)
 Experiment ID: _____ Plate ID: _____

Step 6



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