

# Capturing Single Cell mRNA with the BD Rhapsody™ Single-Cell Analysis System

For detailed instructions and safety information, see the *Single Cell Targeted Library Preparation with the BD Rhapsody™ Single-Cell Analysis System User Guide* (Doc ID: 47395). The ultra-quick reference is intended for expert users.

## Priming and treating the cartridge

Sample Loading Station slider (SLS)	Position
Front	WASTE
Side	0

No.	Reagent to load	Volume (µL)	P1200M Pipette mode	Incubation at room temp.
1	100% ethyl alcohol	700	Prime/Treat	—
2	Air	700	Prime/Treat	—
3	Room temp. Cartridge Wash Buffer 1 (PN 650000060)	700	Prime/Treat	1 min
4	Air	700	Prime/Treat	—
5	Room temp. Cartridge Wash Buffer 1 (PN 650000060)	700	Prime/Treat	10 min
6	Air	700	Prime/Treat	—
7	Room temp. Cartridge Wash Buffer 2 (PN 650000061)	700	Prime/Treat	≤4 h

## Staining cells

No.	Action	Incubation
1	Combine 3.1 µL of 2 mM Calcein AM + 3.1 µL of 0.3 mM Draq7 + 620 µL volume of cell suspension	37°C in dark for 5 min
2	Filter cells through Falcon® Tube with Cell Strainer Cap	—
3	Count 10 µL	—

## Loading cells

No.	Reagent to load	Volume (µL)	P1200M Pipette mode	Incubation at room temp.
1	Prepare cell suspension in ≥650 µL (≥610 µL for low cell number samples) in cold Sample Buffer (PN 650000062)	—	—	—
2	Air	700	Prime/Treat	—
3	Cell suspension immediately after gentle resuspension with P1000 (aspirate 40 µL of air + 575 µL of cells)	615	Cell Load	15 min

Scan	Cell Load
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## Loading and washing Cell Capture Beads

No.	Reagent to load	Volume (µL)	P1200M Pipette mode	Incubation at room temp.
1	Resuspend beads in 750 µL cold Sample Buffer	—	—	—
2	Air	700	Prime/Treat	—
3	Beads immediately after gentle resuspension with P1000	630	Bead Load	3 min

Scan	Bead Load
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No.	Reagent to load	Volume (µL)	P1200M Pipette mode	Incubation
1	Air	700	Wash	—
2	Cold Sample Buffer	700	Wash	—
3	Air	700	Wash	—
4	Cold Sample Buffer	700	Wash	—

Scan	Bead Wash
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## Lysing cells

No.	Action	Incubation
1	Add 75 µL of 1 M DTT to 15 mL Lysis Buffer bottle	—

SLS Slider	Position
Front	WASTE
Side	LYSIS

No.	Reagent to load	Volume (µL)	P1200M Pipette mode	Incubation at room temp.
1	Cold Lysis Buffer with DTT	550	Lysis	2 min

## Retrieving beads

SLS slider	Position
Front	BEADS
Side	RETRIEVAL for 30 s → 0 position

No.	Reagent to load	Volume (µL)	P5000M Pipette mode	Incubation
1	Lysis Buffer with DTT	4,950	Retrieval	—

No.	Action	Incubation at room temp.
1	Place 5 mL LoBind tube on magnet	1 min

Scan	Retrieval
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No.	Action	Incubation
1	Proceed to reverse transcription and Exo I treatment	—
2	Clean SLS with 10% bleach or 70% ethyl alcohol	—

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