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

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

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

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

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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