

Prepare the Reagent Mixes for mRNA Seq Chemistry on Polaris

For more information, see the Polaris mRNA Seq protocol (PN 101-0082).

IMPORTANT

- Thaw all reagents on ice except Loading Reagent, which must be thawed at room temperature and vortexed thoroughly prior to use.
- Do not vortex **Cell Lysis Mix**.
- Keep final mixes on ice.
- Use each mix within 4 hours of its preparation.

Dilution of ArrayControl RNA Spikes for Cell Lysis Mix

Reagent	Storage	Volume (μL)	
Loading Reagent	-20 °C	96.5	<input type="checkbox"/>
SMARTer [®] Kit RNase Inhibitor, 40 U/μL	-20 °C	2.5	<input type="checkbox"/>
Mix the above reagents thoroughly. If including the ArrayControl RNA Spikes, add the following:			<input type="checkbox"/>
(Optional) ArrayControl RNA Spikes Mix	-80 °C	1.0	<input type="checkbox"/>
Total		100.0	

Dilution of Lysis Plus Reagent for Cell Lysis Mix

Reagent	Storage	Volume (μL)	
PCR-Grade Water	-20 °C	199	<input type="checkbox"/>
Polaris [™] Lysis Plus Reagent	-20 °C	1.0	<input type="checkbox"/>
Total		200.0	

Cell Lysis Mix

Reagent	Storage	Volume (μL)	
Polaris Lysis Reagent	-20 °C	8.0	<input type="checkbox"/>
Polaris Lysis Plus Reagent, diluted 200-fold in PCR-Grade Water immediately before use	On ice	9.6	<input type="checkbox"/>
SMARTer Kit, 3' SMART [™] CDS Primer IIA (Takara Bio, Inc.)	-20 °C	9.0	<input type="checkbox"/>
Either (a) Diluted ArrayControl RNA Spikes Mix or (b) the mix prepared only with Loading Reagent and SMARTer Kit RNase Inhibitor.	On ice	1.4	<input type="checkbox"/>
Total		28.0	

Reverse Transcription (RT) Mix

Reagent	Storage	Volume (μL)	
SMARTer Kit 5X First-Strand Buffer (RNase-free) (Takara Bio, Inc.)	-20 °C	15.5	<input type="checkbox"/>
SMARTer Kit Dithiothreitol (Takara Bio, Inc.)	-20 °C	1.9	<input type="checkbox"/>
SMARTer Kit dNTP Mix (dATP, dCTP, dGTP, and dTTP, each at 10 mM) (Takara Bio, Inc.)	-20 °C	7.7	<input type="checkbox"/>
SMARTer Kit SMARTer IIA Oligonucleotide (Takara Bio, Inc.)	-80 °C	7.7	<input type="checkbox"/>
SMARTer Kit RNase Inhibitor (Takara Bio, Inc.)	-20 °C	1.9	<input type="checkbox"/>
SMARTer Kit SMARTscribe [™] Reverse Transcriptase (Takara Bio, Inc.)	-20 °C	7.7	<input type="checkbox"/>
Loading Reagent (thawed completely at room temperature and vortexed thoroughly)	-20 °C	2.4	<input type="checkbox"/>
Polaris RT Plus Reagent	-20 °C, Protect from light	3.2	<input type="checkbox"/>
Total		48.0	

PCR Mix

Reagent	Storage	Volume (μL)	
PCR-Grade Water (Advantage 2 PCR Kit)	-20 °C	63.5	<input type="checkbox"/>
10X Advantage 2 PCR Buffer (not SA, short amplicon) (Advantage 2 Kit)	-20 °C	10.0	<input type="checkbox"/>
50X dNTP Mix (Advantage 2 PCR Kit)	-20 °C	4.0	<input type="checkbox"/>
IS PCR primer (Clontech SMARTer Kit)	-20 °C	4.0	<input type="checkbox"/>
50X Advantage 2 Polymerase Mix (Advantage 2 PCR Kit)	-20 °C	4.0	<input type="checkbox"/>
Loading Reagent (thawed completely at room temperature and vortexed thoroughly)	-20 °C	4.5	<input type="checkbox"/>
Total		90.0	

Tube Control Mixes

NTC is defined as No Template Control.

Lysis Reaction

Reagent	Tube 1: Positive Control (μL)	Tube 2: NTC (μL)	
Prepared cells	1.0	--	<input type="checkbox"/>
Cell Wash Buffer	--	1.0	<input type="checkbox"/>
Cell Lysis Mix	4.5	4.5	<input type="checkbox"/>
Total	5.5	5.5	

Lysis Thermal Cycler Parameters

Temperature	Time
37 °C	5 min
72 °C	3 min
25 °C	1 min
4 °C	Hold

RT Reaction

Reagent	Tube 1: Positive Control (μL)	Tube 2: NTC (μL)	
Lysis reaction	5.5	5.5	<input type="checkbox"/>
RT Mix	9.0	9.0	<input type="checkbox"/>
Total	14.5	14.5	

RT Thermal Cycler Parameters

Temperature	Time
42 °C	90 min
70 °C	10 min
4 °C	Hold

PCR Reaction

Reagent	Tube 1: Positive Control (μL)	Tube 2: NTC (μL)	
PCR Mix	9.3	9.3	<input type="checkbox"/>
RT reaction	1.0	1.0	<input type="checkbox"/>
Total	10.3	10.3	

PCR Thermal Cycler Parameters

Temperature	Time	Cycles
95 °C	1 min	1
95 °C	20 sec	5
58 °C	4 min	
68 °C	6 min	
95 °C	20 sec	9
64 °C	30 sec	
68 °C	6 min	
95 °C	30 sec	7
64 °C	30 sec	
68 °C	7 min	
72 °C	10 min	1
4 °C	Hold	1

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