QUICK PROTOCOL

Ovation® RNA Amplification System V2

Part No. 3100

Enter the following information to automatically calculate the volumes needed to prepare each reaction. The calculated volumes include an appropriate overfill in excess of the nominal volume requirements to allow for volume loss due to handling. Simply print this document to create a working guide for your experiment, which can be kept as a record.

Operator's Name:	**Date:**
Kit Part No:	**Kit Lot No**:
Number of Samples*:	

THERMAL CYCLER PROGRAMS

First Strand cDNA Synthesis	
Program 1: Primer Annealing	65 °C – 5 min, hold at 4 °C
Program 2: First Strand Synthesis	48 °C – 60 min, 70 °C – 15 min, hold at 4 °C
Second Strand cDNA Synthesis	
Program 3: Second Strand Synthesis	37 °C – 30 min, 75 °C – 15 min, hold at 4 °C
SPIA® Amplification & Modification	
Program 4: SPIA Amplification	48 °C – 60 min, 95 °C – 5 min, hold at 4 °C

*Number of samples field ties into embedded logic to calculate master mix volumes, number of reactions.

FIRST STRAND cDNA SYNTHESIS

Obtain **Nuclease-free Water D1 (green)** from -20 °C and leave at room temp.			
Thaw the **First Strand Reagents (blue)**. Mix each reagent, spin and place on ice.			
For each assay place 5 µL of total RNA into 0.2 mL PCR tube and place on ice.			
Add 2 µL of First Strand Primer Mix **A1**, flick tubes to mix and spin.			
Place the tubes in a thermal cycler running Program 1 (65 °C – 5 min, hold at 4 °C).			
After 5 minutes at 65 °C, immediately snap cool tubes on ice.			
Prepare **First Strand Master Mix** (calculation allows for appropriate overfill). Please be sure to pipet **A3** enzyme slowly and rinse out tip at least five times into buffer. Per sample combine: 12 µL Buffer Mix **A2 VER 4** + 1 µL Enzyme Mix **A3 VER 7**. **Mix well.**	**No. of Samples**	**A2**	**A3**
	1	12 µL	1 µL
	0	0.0	0.0
Add 13 µL of the **First Strand Master Mix** to each tube, mix and spin.			
Place the tubes in a thermal cycler running Program 2 (48 °C – 60 min, 70 °C – 15 min, hold at 4 °C).			

SECOND STRAND cDNA SYNTHESIS

Thaw the **Second Strand Reagents (yellow)**. Mix each reagent, spin and place on ice.			
Once the thermal cycler reaches 4 °C, remove tubes, spin and place on ice.			
Prepare **Second Strand Master Mix** (calculation allows for appropriate overfill). Per sample combine: 18 µL Buffer Mix **B1 VER 4** + 2 µL Enzyme Mix **B2 VER 1**. **Mix well.**	**No. of Samples**	**B1**	**B2**
	1	18 µL	2 µL
	0	0.0	0.0
Add 20 µL of **Second Strand Master Mix** to each first strand reaction tube, mix and spin.			
Place the tubes in a thermal cycler running Program 3 (37 °C – 30 min, 75 °C – 15 min, hold at 4 °C).			
Once the thermal cycler reaches 4 °C, spin and place tubes on ice.			

SPIA AMPLIFICATION

Thaw the **SPIA Amplification Reagents (red)**. Vortex **C1** and **C2**, invert **C3** 5 times. Spin all, place on ice.					
Make **SPIA Master Mix** (calculation allows for appropriate overfill). Please be sure to pipet **C3** enzyme slowly and rinse out tip at least five times into buffer. Per sample combine: 72 µL **C2 VER 6** + 4 µL **C1 VER 1** + 4 µL **D1** (water) + 40 µL **C3 VER 5**. **Mix well.**	**No. of Samples**	**C2**	**C1**	**Water D1**	**C3**
	1	72 µL	4 µL	4 µL	40 µL
	0	0	0	0	0
On ice, add 120 µL of **SPIA Master Mix** to each second strand reaction tube, mix and spin.					
Place half of the 160 µL reaction into a separate 0.2 mL PCR tube, cap tightly and spin.					
Place tubes in a thermal cycler running Program 4 (48 °C – 60 min, 95 °C – 5 min, hold at 4 °C).					
Once the thermal cycler reaches 4 °C, spin and place tubes on ice.					
Proceed immediately to purification step or store SPIA cDNA at –20 °C.					

PURIFICATION OF AMPLIFIED SPIA cDNA

Refer to the user guide and follow the method of choice for purification:	**Purification Kit Part No.**	**Purification Kit Lot No.**
Add Binding Buffer in volume of:	Spin at speed:	For a duration of:
Add Wash Buffer in volume of:	Spin at speed:	For a duration of:
Repeat for second wash.		
To elute sample use **Nuclease-free Water D1** provided with the kit.		
Add **Nuclease-free Water D1** in volume of:	Spin at speed:	For a duration of:

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