

Ovation® Pico WTA System

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: _____

Ovation Kit Part No. _____ Ovation Kit Lot No. _____

Number of Samples:* _____

Thermal Cycler Programs	
FIRST STRAND cDNA SYNTHESIS	
Program 1: Primer Annealing	65°C – 2 min, hold at 4°C
Program 2: First Strand Synthesis	4°C – 1 min, 25°C – 10 min, 42°C – 10 min, 70°C – 15 min, hold at 4°C
SECOND STRAND cDNA SYNTHESIS	
Program 3: Second Strand Synthesis	4°C – 1 min, 25°C – 10 min, 50°C – 30 min, 70°C – 5 min, hold at 4°C
SPIA AMPLIFICATION & MODIFICATION	
Program 4: SPIA® Amplification	4°C – 1 min, 47°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			
Thaw the First Strand Reagents (blue) . Mix each reagent, spin and place on ice.			
Add 2 μ L of First Strand Primer Mix A1 to each tube.			
For each sample place 5 μ L of total RNA into 0.2 mL PCR tube, mix and spin.			
Place the tubes in a thermal cycler running Program 1 (65°C – 2 min, hold at 4°C).			
After the cycler reaches 4°C, remove tubes and place 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: 2.5 μ L Buffer Mix A2 VER 3 + 0.5 μ L Enzyme Mix A3 VER 1 . Mix well.	No. of Samples	A2	A3
	1	2.5 μ L	0.5 μ L
Add 3 μ L of the First Strand Master Mix to each tube, mix and spin.			
Place the tubes in a thermal cycler running Program 2 (4°C – 1 min, 25°C – 10 min, 42°C – 10 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: 9.75 μ L Buffer Mix B1 VER 3 + 0.25 μ L Enzyme Mix B2 VER 2 . Mix well.	No. of Samples	B1	B2
	1	9.75 μ L	0.25 μ L
Add 10 μ L of Second Strand Master Mix to each first strand reaction tube, mix and spin.			
Place the tubes in a thermal cycler running Program 3 (4°C – 1 min, 25°C – 10 min, 50°C – 30 min, 70°C – 5 min, hold at 4°C).			
Once the thermal cycler reaches 4°C, spin and place tubes on ice.			

Purification of Double-Stranded cDNA	
Resuspend the RNAClean® beads and bring to room temperature.	RNAClean Bead Lot No.
Remove reaction tubes from ice and place on bench at room temperature.	
Add 32 µL of beads to each reaction and mix.	
Incubate at room temperature for 10 minutes.	
Place tubes on magnet for 5 minutes to completely clear the beads.	
To minimize bead loss, remove only 45 µL of Binding Buffer before the first wash step.	
Wash the beads while still on the magnet for 30 seconds with 200 µL of freshly prepared 70% ethanol. Repeat wash 2 more times.	
Dry beads completely, at least for 15 to 20 minutes.	
Proceed with SPIA amplification.	

SPIA Amplification				
Thaw the SPIA Amplification Reagents (red) . Vortex C1 and C2 , invert C3 5 times. Spin all, place on ice.				
Prepare SPIA Master Mix (calculation allows for appropriate overfill). Per sample combine: 80 µL C2 VER 5 + 40 µL C1 VER 4 + 40 µL C3 VER 5 . Mix well.	No. of Samples	C2	C1	C3
	1	80 µL	40 µL	40 µL
On ice, add 160 µL of SPIA Master Mix to each second strand reaction tube containing the dried beads, 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 (4°C – 1 min, 47°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 Ovation Kit.		
Add Nuclease-Free Water D1 in volume of:	Spin at speed:	For a duration of: