Ovation® Pico WTA System V2

Part No. 3302

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 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 – 2 min, 25 °C – 30 min, 42 °C – 15 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, 80 °C – 20 min, hold at 4 °C
SPIA AMPLIFICATION & MODIFICATION	
Program 4: SPIA® Amplification	4 °C – 1 min, 47 °C – 75 min, 95 °C – 5 min, hold at 4 °C

First Strand cDNA Synthesis

Thaw the **First Strand Reagents (blue)**. Mix each reagent, spin and place on ice.

For each sample, place 2 µL of First Strand Primer Mix **A1 VER 8** into a 0.2 mL PCR tube.

Add 5 µL of total RNA to each 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). Pipet **A3** enzyme slowly and rinse out tip at least five times into buffer. Per sample combine:
2.5 µL **A2 VER 3** + 0.5 µL **A3 VER 1**.
Mix well.

No. of Samples	A2	A3
1	2.5 µL	0.5 µL
0	0	0

Add 3 µL of the **First Strand Master Mix** to each tube, mix and spin.

Place the tubes in a thermal cycler running Program 2
(4 °C – 2 min, 25 °C – 30 min, 42 °C – 15 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:
9.7 µL **B1 VER 3** + 0.3 µL **B2 VER 2**.
Mix well.

No. of Samples	B1	B2
1	9.7 µL	0.3 µL
0	0	0

Add 10 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
(4 °C – 1 min, 25 °C – 10 min, 50 °C – 30 min, 80 °C – 20 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 Agencourt® beads and bring to room temperature.	**Agencourt 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: 50 µL **C2** VER **10** + 25 µL **C1** VER **9** + 25 µL **C3** VER **7**. **Mix well.**	**No. of Samples**	**C2**	**C1**	**C3**
	1	50 µL	25 µL	25 µL
	0	0	0	0
On ice, add 100 µL of **SPIA Master Mix** to each second strand reaction tube containing the dried beads, mix and spin.				
Place tubes in a thermal cycler running Program 4 (4 °C – 1 min, 47 °C – 75 min, 95 °C – 5 min, hold at 4 °C).				
Once the thermal cycler reaches 4 °C, spin and place tubes on ice.				
For Agencourt bead purification of SPIA cDNA, proceed immediately to **"Bead-based Purification of Amplified SPIA cDNA"**				
Transfer the tubes to the magnet and let stand for 5 minutes to completely clear the solution of beads.				
Carefully remove all of the cleared supernatant containing the eluted SPIA cDNA and transfer to a fresh tube.				
Proceed immediately to purification step or store SPIA cDNA at –20 °C.				

Column-based 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:

Bead-based Purification of Amplified SPIA cDNA
Bring to room temperature and resuspend the beads. Prepare 80% ethanol.
Remove reaction tubes from ice and place on bench at room temperature.
Split the 100 µL reaction into two 50 µL aliquots. The two half-reactions must be in separate strip-tube or 96-well plates.
Add 90 µL of beads to one set of the half-reactions. Mix by pipetting 10 times.
Incubate at room temperature for 5 minutes.
Place the sample/bead mixture on the magnet for 10 minutes to completely clear the solution.
After 5 minutes of the 10 minute incubation, add 90 µL of beads to the second half-reaction and mix by pipetting 10 times. Incubate for 5 additional minutes.
Keeping the first tube on the magnet, carefully remove and discard the supernatant from the first set of samples. Do not disturb the beads. Keep samples on the magnet.
With the first tube on the magnet, add the sample/bead mixture from the second half-reaction to the tube containing the first half-reaction. Add slowly so as to not disturb the beads already in the tube.
Incubate additional 10 minutes to completely clear the solution of beads.
Carefully remove and discard the supernatant.
Wash the beads while still on the magnet for 30 seconds with 200 µL of freshly prepared 80% ethanol. Repeat wash 1 more time.
Carefully remove and discard the supernatant. Dry beads for 2 min.
Add 30 µL of room temperature **Nuclease-free Water D1** to the tube. Resuspend the beads by pipetting or vortexing.
Place the tubes on the magnet and incubate at room temperature for 5 minutes to completely clear the solution.
Carefully remove the eluted sample and transfer to a fresh tube.
Continue with the Measuring SPIA cDNA Yield and Purity protocol or store the purified SPIA cDNA at –20 °C.