

# WT-Ovation™ FFPE System V2

Enter the following information to automatically calculate the volumes needed to prepare each reaction. Simply print this page and record the essential information for this experiment.

Operator's Name: \_\_\_\_\_ Date: \_\_\_\_\_

WT-Ovation Kit Cat. No. \_\_\_\_\_ WT-Ovation Kit Lot No. \_\_\_\_\_

SPIA Product Purification Kit Name / Lot No. \_\_\_\_\_ Number of Samples:\* \_\_\_\_\_

First Strand cDNA Synthesis			
Obtain <b>Nuclease-Free Water D1</b> (green cap vial) from -20°C and leave at room temp.			
Thaw the <b>First Strand Reagents</b> (Set A, blue cap vials). Mix each reagent, spin and place on ice.			
For each assay, place 2 µL of First Strand Primer Mix <b>A1</b> into a 0.2 mL PCR tube and place on ice.			
Add 5 µL of total RNA (50 ng) to the primer, flick tubes to mix and spin.			
Place the tubes in a thermal cycler running Program 1 (65°C – 2 min, hold at 4°C). When cycler reaches 4°C, spin and place tubes on ice.			
Make <b>First Strand Master Mix</b> . Per sample combine: 2.5 µL Buffer Mix <b>A2</b> + 0.5 µL Enzyme Mix <b>A3</b> .	<b>No. of Samples</b>	<b>A2</b>	<b>A3</b>
	1	2.5 µL	0.5 µL
Enter your number of samples and volumes of each reagent:			
Mix the <b>First Strand Master Mix</b> , spin and place on ice.			
Add 3 µL of the <b>First Strand Master Mix</b> 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).			
Once the thermal cycler reaches 4°C, spin and place tubes on ice.			

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

### Second Strand cDNA Synthesis

Resuspend the RNAClean® beads provided with the WT-Ovation kit and leave at room temperature.

Thaw the **Second Strand Reagents** (Set B, **yellow** cap vials). Mix each reagent, spin and place on ice.

Make **Second Strand Master Mix**. Per sample combine:  
9.75 µL Buffer Mix **B1** + 0.25 µL Enzyme Mix **B2**.

No. of Samples	B1	B2
1	9.75 µL	0.25 µL

Enter your number of samples and volumes of each reagent:

Mix the **Second Strand Master Mix**, spin and place on ice.

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, 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

Ensure the magnetic bead suspension has reached room temperature.

Mix the bead suspension by inverting several times.

At room temperature, add 32 µL of the bead suspension to each tube, mix and incubate at room temperature for 10 min.

Transfer samples to magnet, let stand for 5 min, remove 45 µL of the binding buffer. Minimize bead loss at this step.

Add 200 µL of **freshly prepared** 70% ethanol, let stand for 30 seconds, then remove the ethanol using a pipette.

Repeat the ethanol wash 2 times, remove all excess ethanol and let beads air dry for 15-20 minutes.

Ensure all residual ethanol is removed from the sample.

Proceed immediately with SPIA® amplification, with the cDNA bound to the dry beads.

SPIA Amplification					
Thaw the <b>SPIA Amplification Reagents</b> (Set C, <b>red</b> cap vials). Vortex <b>C1</b> , <b>C2</b> and <b>C5</b> . Gently pipette mix <b>C3</b> and <b>C6</b> . Spin, place on ice.					
Make <b>SPIA Master Mix 1</b> . Per sample combine: 50 $\mu$ L <b>C2</b> + 20 $\mu$ L <b>C1</b> + 0.7 $\mu$ L <b>C6</b> , mix and then add 10 $\mu$ L <b>C3</b> . Mix well.	<b>No. of Samples</b>	<b>C2</b>	<b>C1</b>	<b>C6</b>	<b>C3</b>
	1	50 $\mu$ L	20 $\mu$ L	0.7 $\mu$ L	10 $\mu$ L
Enter your number of samples and volumes of each reagent:					
Mix <b>SPIA Master Mix 1</b> , spin and place on ice.					
On ice, add 80 $\mu$ L of <b>SPIA Master Mix 1</b> to each second strand reaction tube, mix and spin.					
Place tubes in a thermal cycler running Program 4 (4°C – 1 min, 47°C – 30 min, hold at 4°C).					
Once the thermal cycler reaches 4°C, spin and place tubes on ice.					
Make <b>SPIA Master Mix 2</b> . Per sample combine: 30 $\mu$ L <b>C2</b> + 20 $\mu$ L <b>C5</b> + 2.3 $\mu$ L <b>C6</b> , mix and then add 30 $\mu$ L <b>C3</b> . Mix well.	<b>No. of Samples</b>	<b>C2</b>	<b>C5</b>	<b>C6</b>	<b>C3</b>
	1	30 $\mu$ L	20 $\mu$ L	2.3 $\mu$ L	30 $\mu$ L
Enter your number of samples and volumes of each reagent:					
Mix <b>SPIA Master Mix 2</b> , spin and place on ice.					
On ice, add 80 $\mu$ L of <b>SPIA Master Mix 2</b> to each reaction tube and mix, transfer 80 $\mu$ L to a second reaction tube.					
Place tubes in a thermal cycler running Program 5 (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 your method of choice for purification:	<b>Purification Kit Part No.</b>	<b>Purification Kit Lot No.</b>
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 <b>Nuclease-Free Water D1</b> provided with the WT-Ovation kit.		
Add <b>Nuclease-Free Water D1</b> in volume of:	Spin at speed:	For a duration of: