QUICK PROTOCOL

WT-Ovation™ One-Direct System (3500-12)

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: _____

WT-Ovation Kit Part No: _____ WT-Ovation Kit Lot No.: _____

Number of Samples:* _____

Thermal Cycler Programs	
FIRST STRAND cDNA SYNTHESIS	
Program 1: First Strand Primer Annealing	65°C – 2 min, hold at 4°C
Program 2: First Strand Synthesis	4°C – 2 min, 25°C – 30 min, 48°C – 30 min, 95°C – 5 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 – 90 min, 95°C – 5 min, hold at 4°C
POST-SPIA MODIFICATION	
Program 5: Post-SPIA Primer Annealing	95°C – 5 min, hold at 4°C
Program 6: Post-SPIA Modification	4°C – 1 min, 30°C – 10 min, 42°C – 60 min, 75°C – 10 min, hold at 4°C

Cell Lysate Preparation (pre-amplification workspace)	
Obtain and count cells.	
Deliver cells to lysis buffer or add lysis buffer to washed cell pellet. Note: a maximum of 2 µL of lysate can be used in the amplification, so plan accordingly.	
Lyse cells by gently pipetting up and down several times. Take care to minimize foaming at this step.	
Place lysate on ice.	
Proceed immediately with First Strand cDNA Synthesis or store cell lysates at –80°C.	

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

First Strand cDNA Synthesis (note: set up in pre-amplification workspace)	
Thaw the **First Strand Reagents (blue)** and **Nuclease-Free Water D1 (green)**.	
Vortex **A1 VER 5** and **A2 VER 5**. Flick **A3 VER 3** to mix. Spin all. Place on ice.	

IF USING CELL LYSATE

On ice, mix 2 µL lysate, 3 µL water and 2 µL **A1 VER 5** in a 0.2 mL PCR tube.			
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 VER 3** enzyme slowly and rinse out tip at least five times into buffer. Per sample combine: 2.5 µL Buffer Mix **A2 VER 5** + 0.5 µL Enzyme Mix **A3 VER 3**. **Mix well.**	**No. of Samples**	**A2**	**A3**
	1	2.5 µL	0.5 µL
	0	0.0	0.0

IF USING PURIFIED TOTAL RNA

On ice, mix 5 µL total RNA (10+ pg) and 2 µL **A1 VER 5** in a 0.2 mL PCR tube.				
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 VER 3** enzyme slowly and rinse out tip at least five times into buffer. Per sample combine: 2.5 µL Buffer Mix **A2 VER 5** + 0.25 µL Enzyme Mix **A3 VER 3** + 0.25 µL **D1**. **Mix well.**	**No. of Samples**	**A2**	**A3**	**D1**
	1	2.5 µL	0.25 µL	0.25 µL
	0	0.0	0.0	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, 48°C – 30 min, 95°C – 5 min, hold at 4°C).	
Proceed immediately to Second Strand cDNA Synthesis.	

Second Strand cDNA Synthesis (pre-amplification workspace)

Remove Agencourt® RNAClean® beads from 4°C and allow to come to room temperature for use in the following step.

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). Please be sure to pipet **B2 VER 2** enzyme slowly. Do not rinse out tip. Per sample combine:
9.6 µL Buffer Mix **B1 VER 3** + 0.4 µL Enzyme Mix **B2 VER 2**.
Mix well.

No. of Samples	B1	B2
1	9.6 µL	0.4 µL
0	0.0	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.

Proceed immediately to Purification of Double-stranded cDNA.

Purification of Double-stranded cDNA (pre-amplification workspace)

Resuspend the RNAClean beads and ensure they have reached room temperature.

Remove reaction tubes from ice and place on bench at room temperature.

Prepare a 70% ethanol solution. Measure each component. Make fresh wash solution each day from recently opened 100% ethanol stock.

At room temperature, add 28 µl of the bead mix to each tube, mix by pipetting up and down 10 times.

Incubate at room temperature for 20 minutes.

Transfer samples to magnet and let stand for 5 minutes. Remove only 40 µL of the buffer and discard in order to minimize bead loss.

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

Repeat the ethanol wash an additional 2 times for a total of 3 washes. After final wash, remove as much of the ethanol as possible.

Dry the beads for 15 to 20 minutes until completely dry.

Proceed immediately to SPIA Amplification with the cDNA bound to the dry beads.

SPIA Amplification (pre-amplification workspace)

Thaw the **SPIA Amplification Reagents (red)**. Vortex **C2 VER 5** and **C1 VER 7**, invert **C3 VER 5** 5 times. Spin all, place on ice.

Prepare **SPIA Master Mix** (calculation allows for appropriate overfill).
Per sample combine:
75 µL **C2 VER 5** + 37.5 µL **C1 VER 7** + 37.5 µL **C3 VER 5**.
Mix well.

No. of Samples	C2	C1	C3
1	75 µL	37.5 µL	37.5 µL
0	0	0	0

On ice, add 150 µL of the **SPIA Master Mix** to each second strand reaction tube. Mix carefully with pipette, resuspending the RNAClean beads.

Transfer one half of the reaction volume (75 µL) to a second reaction tube.

Place tubes in a thermal cycler running Program 4
(4°C – 1 min, 47°C – 90 min, 95°C – 5 min, hold at 4°C).

Once the thermal cycler reaches 4°C, spin and place tubes on ice. Tubes should not be opened again in the pre-amplification workspace.

Proceed immediately to Post-SPIA Modification or store SPIA cDNA at –20°C.

Post-SPIA Modification (note: move to post-amplification workspace for the remainder of the protocol)			
Thaw the **Post-SPIA Modification Reagents (violet)**. Vortex **E1 VER 1** and **E2 VER 2**. Invert **E3 VER 1** 3 times. Spin all. Place on ice.			
Place tubes containing the SPIA half-reactions on the SPRIPlate Super Magnet Plate and let stand 5 minutes.			
Add 70 µL of the supernatant to each of two fresh reaction tubes. If there is insufficient volume due to evaporation, add water.			
Add 10 µL **E1 VER 1** Primer mix to each of the two half-reaction tubes. Mix well by pipetting.			
Place tubes in a thermal cycler running Program 5 (95°C – 5 min, hold at 4°C).			
Once the thermal cycler reaches 4°C, remove tubes and place on ice.			
Prepare **Post-SPIA Modification Master Mix** (calculation allows for appropriate overfill). Per sample combine: 20 µL **E2 VER 2** + 20 µL **E3 VER 1** **Mix well.** **Note:** the master mix is prepared per sample, not per sub-reaction.	**No. of Samples**	**E2**	**E3**
	1	20 µL	20 µL
	0	0	0
On ice, add 20 µL of the **Post-SPIA Modification Master Mix** to each of the two half-reaction tubes. Mix well by pipetting.			
Place tubes in a thermal cycler running Program 6 (4°C – 1 min, 30°C – 10 min, 42°C – 60 min, 75°C – 10 min, hold at 4°C).			
Once the thermal cycler reaches 4°C, spin and place tubes on ice.			
Proceed immediately to Purification of SPIA cDNA or store SPIA cDNA at –20°C			

Purification of Amplified SPIA cDNA (post-amplification workspace)		
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:
Number of washes:		
To elute sample use **Nuclease-free Water D1** provided with the WT-Ovation Kit.		
Add **Nuclease-free Water D1** in volume of:	Spin at speed:	For a duration of:

NuGEN®
imagine more from less™