Applause® WT-Amp Plus ST System (Part No. 5510)

Enter the number of reactions you are running in the provided field to automatically calculate the volumes needed to prepare each master mix. 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: _____

Applause WT-Amp Plus ST System Part No.: _____ Lot No.: _____

Number of Samples:* 10

Thermal Cycler Programs	
FIRST STRAND cDNA SYNTHESIS	
Program 1: Primer Annealing	65°C – 5 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
POST-SECOND STRAND ENHANCEMENT	
Program 4: Post-Second Strand Enhancement	4°C – 1 min, 37°C – 15 min, 80°C – 20 min, hold at 4°C
SPIA® AMPLIFICATION	
Program 5: SPIA Amplification	4°C – 1 min, 47°C – 90 min, 95°C – 5 min, hold at 4°C
POST-SPIA MODIFICATION	
Program 6: Post-SPIA Modification I	4°C – 1 min, 37°C – 15 min, 95°C – 5 min, hold at 4°C
Program 7: Post-SPIA Modification II	4°C – 1 min, 30°C – 10 min, 42°C – 60 min, 75°C – 10 min, hold at 4°C
BIOTIN LABELING	
Program 8: cDNA Fragmentation	37°C – 30 min, 95°C – 2 min, hold at 4°C
Program 9: Biotin Labeling	37°C – 60 min, 70°C – 10 min, hold at 4°C

** Number of samples field ties into embedded logic used to calculate suggested master mix volumes. NuGEN recommends processing a minimum of 8 samples at a time.*

First Strand cDNA Synthesis			
Thaw the **First Strand cDNA Synthesis Reagents (blue)** and **Nuclease-free Water (green)**.			
Spin **A3 VER 1** briefly and place on ice. Vortex **A1 VER 4** and **A2 VER 3**, spin and place on ice. Leave **Nuclease-free Water** at room temperature.			
On ice, mix 5 µL total RNA (50 ng to 200 ng) and 2 µL Primer Mix **A1** in a PCR tube.			
Place the tubes in a thermal cycler running Program 1 (65°C – 5 min, hold at 4°C).			
Once the thermal cycler reaches 4°C, remove tubes and place on ice.			
Prepare **First Strand Master Mix** (calculated volumes allow for appropriate overfill). Be sure to pipet **A3** enzyme slowly and rinse out tip at least five times into buffer. Per sample combine: 2.5 µL Buffer Mix **A2 VER 3** + 0.5 µL Enzyme Mix **A3 VER 1**. **Mix well.**	**No. of Samples**	**A2**	**A3**
	1	2.5 µL	0.5 µL
	10	27.50	5.50
Add 3 µL of **First Strand Master Mix** to each tube, mix by pipetting, spin and place on ice.			
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).			
Once the thermal cycler reaches 4°C, remove tubes, spin and place on ice.			
Continue immediately with Second Strand cDNA Synthesis.			

Second Strand cDNA Synthesis			
Thaw the **Second Strand cDNA Synthesis Reagents (yellow)**.			
Spin **B2 VER 2** and **B3 VER 1** briefly and place on ice. Vortex **B1 VER 3**, spin and place on ice.			
Prepare **Second Strand Master Mix**. Please be sure to pipet **B2** enzyme slowly and rinse out tip at least five times into buffer. Per sample combine: 9.75 µL Buffer Mix **B1 VER 3** + 0.25 µL Enzyme Mix **B2 VER 2**. **Mix well.**	**No. of Samples**	**B1**	**B2**
	1	9.75 µL	0.25 µL
	10	107.25	2.75
Add 10 µL of **Second Strand Master Mix** to each reaction tube, mix by pipetting, spin and place on ice.			
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, remove tubes, spin and place on ice.			
Continue immediately with Post-Second Strand Enhancement.			

Post-Second Strand Enhancement

Prepare **Enhancement Master Mix**. Please be sure to pipet B3 enzyme slowly and rinse out tip at least five times into buffer.

Per sample combine:
3.7 µL Buffer Mix B1 VER 3 + 0.3 µL Enzyme Mix B3 VER 1.
Mix well.

No. of Samples	B1	B3
1	3.7 µL	0.3 µL
10	40.70	3.30

Add 2 µL of **Enhancement Master Mix** to each reaction tube, mix by pipetting, spin and place on ice.
Keep the remaining Enhancement Master Mix on ice for use in the Post-SPIA Modification I step!

Place the tubes in a thermal cycler running Program 4
(4°C – 1 min, 37°C – 15 min, 80°C – 20 min, hold at 4°C).

Once the thermal cycler reaches 4°C, remove tubes, spin and place on ice.

Continue immediately with SPIA Amplification.

SPIA Amplification

Thaw the **SPIA Amplification Reagents (red)**.

Invert **C3 VER 5** 5 times to mix, spin and place on ice. Vortex **C1 VER 8** and **C2 VER 8**, spin and place on ice.

Prepare **SPIA Master Mix**.

Per sample combine:
2.5 µL **C2 VER 8** + 2.5 µL **C1 VER 8** + 5 µL **C3 VER 5**.
Mix well.

No. of Samples	C2	C1	C3
1	2.5 µL	2.5 µL	5 µL
10	27.50	27.50	55.00

Add 10 µL of the **SPIA Master Mix** to each reaction tube, mix thoroughly, spin and place on ice.

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

Once the thermal cycler reaches 4°C, remove tubes, spin and place on ice.

Continue immediately with Post-SPIA Modification I or store reactions at –20°C.

Post-SPIA Modification I			
Thaw the **Post-SPIA Modification Reagents (violet)**.			
Spin **E3 VER 1** briefly and place on ice. Vortex **E1 VER 1** and **E2 VER 2**, spin and place on ice.			
Prepare **Post-SPIA Modification I Master Mix**. Per sample combine: 2 µL **Enhancement Master Mix** + 6 µL **E1 VER 1** **Mix well.**	**No. of Samples**	**Enhancement Master Mix**	**E1**
	1	2 µL	6 µL
	10	22.00	66.00
Add 8 µL of the **Modification I Master Mix** to each reaction tube, mix thoroughly and spin down.			
Place tubes in a thermal cycler running Program 6 (4°C – 1 min, 37°C – 15 min, 95°C – 5 min, hold at 4°C).			
Once the thermal cycler reaches 4°C, remove tubes, spin and place on ice.			
Continue immediately with Post-SPIA Modification II.			

Post-SPIA Modification II			
Prepare **Post-SPIA Modification II Master Mix**. Per sample combine: 6 µL **E2 VER 2** + 6 µL **E3 VER 1** **Mix well.**	**No. of Samples**	**E2**	**E3**
	1	6 µL	6 µL
	10	66.00	66.00
Add 12 µL of the **Post-SPIA Modification II Master Mix** to each reaction tube, mix by pipetting, spin and place on ice.			
Place tubes in a thermal cycler running Program 7 (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, remove tubes, spin and place on ice.			
Continue immediately with Purification of Amplified ST-cDNA or store reactions at –20°C.			

Purification of Amplified ST-cDNA		
Use the QIAGEN® MinElute® Reaction Cleanup Kit as described in the user guide for final ST-cDNA purification.	**Kit Part No.**	**Kit Lot No.**
	#28204	
Mix 300 µL Buffer ERC with 52 µL of amplified ST-cDNA, vortex and spin down.		
Load the entire volume of sample/buffer mix onto the MinElute column.		
Spin for 1 minute at maximum speed. Discard the flow-through.		
Add 750 µL of Buffer PE (with EtOH added) to the column.		
Spin for 1 minute at maximum speed. Discard the flow-through.		
Spin for 2 minutes at maximum speed. Discard the collection tube and flow-through.		
Place the column into a clean labeled 1.5 mL microcentrifuge tube.		
Add 15 µL of **Nuclease-free Water**.		
Wait 1 minute. Spin for 1 minute at maximum speed.		
Discard the column and measure the volume recovered. There should be approximately 13 µL of purified ST-cDNA.		
Continue with the Measuring cDNA Product Yield and Purity protocol in the user guide or store purified ST-cDNA at –20°C.		

cDNA Fragmentation			
Thaw the **Encore® Biotin Module Reagents (orange: FL1–FL5)**.			
Spin **FL2** and **FL5** briefly and place on ice. Vortex **FL1**, **FL3** and **FL4**, spin and place on ice.			
Add 25 µL of ST-cDNA (4 to 5 µg) to a 0.2 mL PCR tube.			
Prepare **Fragmentation Master Mix**.	**No. of Samples**	**FL1**	**FL2**
Per sample combine: 5 µL Fragmentation Buffer Mix **FL1** + 2 µL Fragmentation Enzyme Mix **FL2**. **Mix well.**	**1**	5 µL	2 µL
	10	55.00	22.00
Add 7 µL of **Fragmentation Mix** to each tube, mix thoroughly by pipetting, spin and place on ice.			
Place the tubes in a thermal cycler running Program 8 (37°C – 30 min, 95°C – 2 min, hold at 4°C).			
Once the thermal cycler reaches 4°C, remove tubes, spin and place on ice.			
Continue immediately with Biotin Labeling.			

Biotin Labeling				
Prepare **Labeling Master Mix**.	**No. of Samples**	**FL3**	**FL4**	**FL5**
Per sample combine: 15 µL Labeling Buffer Mix **FL3** + 1.5 µL Labeling Reagent **FL4** + 1.5 µL Labeling Enzyme Mix **FL5**. **Mix well.**	**1**	15 µL	1.5 µL	1.5 µL
	10	165.00	16.50	16.50
Add 18 µL of **Labeling Master Mix** to each tube, mix thoroughly by pipetting, spin and place on ice.				
Place the tubes in a thermal cycler running Program 9 (37°C – 60 min, 70°C – 10 min, hold at 4°C).				
Once the thermal cycler reaches 4°C, remove tubes, spin and place on ice.				
Store labeled ST-cDNA at –20°C or use immediately for array hybridization as described in the Applause WT-Amp Plus ST System user guide.				

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