

Applause™ WT-Amp ST / WT-Amp Plus ST Systems (5500-24/5510-24)

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: _____

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

SPIA® cDNA Purification Method and Lot No.: _____ Number of Samples:* _____

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 & MODIFICATION	
Program 5: SPIA® Amplification	4°C – 1 min, 47°C – 90 min, 95°C – 5 min, hold at 4°C
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

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

NuGEN recommends processing a minimum of 8 samples at a time.

First Strand cDNA Synthesis (process is the same for WT-Amp ST and WT-Amp Plus ST)			
Thaw the First Strand Reagents (blue) and Nuclease-Free Water (green) .			
Vortex A1 and A2 . Flick A3 to mix. Spin all. Place on ice.			
On ice, mix 5 μ L total RNA (50 ng to 200 ng) and 2 μ L A1 in a PCR tube.			
Place the tubes in a thermal cycler running Program 1 (65°C – 5 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 (process is the same for WT-Amp ST and WT-Amp Plus ST)			
Thaw the Second Strand Reagents (yellow) . Vortex B1 . Flick B2 to mix. Spin all 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 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
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.			

Post-Second Strand Enhancement			
Prepare Post-Second Strand Enhancement Master Mix (calculation allows for appropriate overfill). 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
Add 2 µL of Post-Second Strand Enhancement Master Mix to each Second Strand reaction tube, mix carefully by pipetting and spin. Important! Keep the remaining Enhancement Master Mix on ice for use in a later 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, spin and place tubes on ice.			
Continue immediately with SPIA Amplification.			

Please note that the WT-Amp ST and WT-Amp Plus ST protocols diverge at this point.

SPIA Amplification & Modification — WT-Amp ST System				
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: 1.25 µL C2 VER 8 + 1.25 µL C1 VER 8 + 2.5 µL C3 VER 5 . Mix well.	No. of Samples	C2	C1	C3
	1	1.25 µL	1.25 µL	2.5 µL
Add 5 µL of the SPIA Master Mix to each reaction tube, mix thoroughly and spin down.				
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, spin and place tubes on ice.				

Post-SPIA Modification I/II—WT-Amp ST System			
Thaw the Post-SPIA Modification Reagents (violet) . Vortex E1 and E2 . Invert to mix E3 . Spin all. Place on ice.			
Prepare Post-SPIA Modification I Master Mix (calculation allows for appropriate overfill). Per sample combine: 2 µL Enhancement Master Mix + 4 µL E1 VER 1 Mix well.	No. of Samples	Enhancement Master Mix	E1
	1	2 µL	4 µL
Add 6 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, spin and place tubes on ice.			
Prepare Post-SPIA Modification II Master Mix (calculation allows for appropriate overfill). Per sample combine: 4 µL E2 VER 2 + 4 µL E3 VER 1 Mix well.	No. of Samples	E2	E3
	1	4 µL	4 µL
Add 8 µL of the Modification II Master Mix to each reaction tube, mix thoroughly and spin down.			
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, spin and place tubes on ice. Proceed with cDNA purification as per User Guide.			

SPIA Amplification & Modification — WT-Amp Plus ST System				
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: 2 μ L C2 VER 8 + 2 μ L C1 VER 8 + 4 μ L C3 VER 5 . Mix well.	No. of Samples	C2	C1	C3
	1	2 μ L	2 μ L	4 μ L
Add 8 μ L of the SPIA Master Mix to each reaction tube, mix thoroughly and spin down.				
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, spin and place tubes on ice.				

Post-SPIA Modification I/II—WT-Amp Plus ST System			
Thaw the Post-SPIA Modification Reagents (violet) . Vortex E1 and E2 . Invert to mix E3 . Spin all. Place on ice.			
Prepare Post-SPIA Modification I Master Mix (calculation allows for appropriate overfill). Per sample combine: 2 μ L Enhancement Master Mix + 5 μ L E1 VER 1 Mix well.	No. of Samples	Enhancement Master Mix	E1
	1	2 μ L	5 μ L
Add 7 μ L 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, spin and place tubes on ice.			
Prepare Post-SPIA Modification II Master Mix (calculation allows for appropriate overfill). Per sample combine: 5 μ L E2 VER 2 + 5 μ L E3 VER 1 Mix well.	No. of Samples	E2	E3
	1	5 μ L	5 μ L
Add 10 μ L of the Modification II Master Mix to each reaction tube, mix thoroughly and spin down.			
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, spin and place tubes on ice. Proceed with cDNA purification as per User Guide.			