Applause™ 3′-Amp System (5100-24)

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 3′-Amp System Lot No.: _____

SPIA® Product Purification Kit Name 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, 48°C – 60 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
SPIA AMPLIFICATION	
Program 4: SPIA® Amplification	4°C – 1 min, 47°C – 90 min, 95°C – 5 min, hold at 4°C

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

First Strand cDNA Synthesis

Thaw the **First Strand Reagents (blue)** and **Nuclease Free Water D1 (green).** Mix each reagent, spin and place on ice.

Add 2 µL of **A1** into each 0.2 mL PCR tube.

For each sample place 5 µL of RNA (50–200 ng) into a 0.2 mL PCR tube, mix and spin.

Place the tubes in a thermal cycler running Program 1 (65°C – 5 min, hold at 4°C).

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 µL Buffer Mix **A2** VER **6** + 0.5 µL Enzyme Mix **A3** VER **1**
Mix well.

No. of Samples	A2	A3
1	2.5 µL	0.5 µL
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 – 1 min, 48°C – 60 min, 70°C – 15 min, hold at 4°C)

Second Strand cDNA Synthesis

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:
9.5 µL Buffer Mix **B1** VER **3** + 0.5 µL Enzyme Mix **B2** VER **2**.
Mix well.

No. of Samples	B1	B2
1	9.5 µL	0.5 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, 70°C – 5 min, hold at 4°C).

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

SPIA Amplification

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

Prepare **SPIA Master Mix** (calculation allows for appropriate overfill).
Per sample combine:
36 µL **C2 VER 6** + 4 µL **C1 VER 10** + 20 µL **C3 VER 5**.
Mix well.

No. of Samples	C2	C1	C3
1	36 µL	4 µL	20 µL
0	0	0	0

Add 60 µL of the **SPIA Master Mix** to each Second Strand synthesis reaction tube, mix thoroughly and spin down.

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.

Proceed immediately to purification step or store SPIA cDNA at -20°C.

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 Ovation Kit.		
Add **Nuclease-Free Water D1** in volume of:	Spin at speed:	For a duration of:

NuGEN® imagine more from less™