

Fluidigm® Gene Expression Specific Target Amplification Quick Reference

PN 68000133 Rev C

1 Overview

The BioMark™ System uses a sample loading volume of 5 µL, and distributes this sample mixture across 48 or 96 reaction chambers in 9 or 6 nL aliquots, respectively. With these micro-volumes, detecting the specific targets requires a minimum of 500-1,000 copies in the original 5 µL loading volume. Because some genes exhibit low expression resulting in more dilute target concentrations, we recommend using Specific Target Amplification to increase target concentration.

Specific Target Amplification (STA) uses the TaqMan® PreAmp Master Mix and TaqMan Gene Expression Assays, both from Applied Biosystems. STA allows for a multiplexed preamplification of up to 100 targets by using a 0.2X pool of gene expression assays as the source of primers. By using the same assays in the preamplification reaction as the Real-Time PCR reaction, only the targets of interest are amplified. The 0.2X concentration of primers creates a primer-limited environment that is further limited by the recommended 14 cycles. This results in small amounts of cDNA being amplified equally without introducing bias.

2 Pooling the TaqMan Assays

- In a 0.5 mL microcentrifuge tube, combine equal volumes of each 20X TaqMan Gene Expression assays, up to a total of 100 assays.
- Dilute the pooled assays using DNA Suspension Buffer (10 mM Tris, pH 8.0, 0.1 mM EDTA) (TEKnova, PN T0221) so that each assay is at a final concentration of 0.2X.
- The chart below provides an example using 50 assays:

50 Assays	DNA Suspension Buffer	Total Volume
1 µL each assay (20X)	50 µL	100 µL

NOTE VOLUME CAN BE INCREASED BASED ON THE NUMBER OF SAMPLES TO BE AMPLIFIED.

3 Preparing Sample Pre-Mix and Samples

- 1 Combine the components in the table below to make the Sample Pre-Mix and the final Sample Mixture. (Scale up appropriately for multiple samples.)

SAMPLE PRE-MIX	Reagent	Volume per Reaction (µL)	Volume for 48 Samples (µL) (60 for ease of pipetting)	Volume for 96 Samples (µL) (120 for ease of pipetting)
	TaqMan PreAmp Master Mix (2X) PN 4391128	2.5	150	300
	Pooled assay mix (0.2X)	1.25	75	150
	cDNA	1.25		
	Final Volume	5		

- 2 In a DNA-free hood, combine the TaqMan Pre Amp Master Mix with the pooled assay mix in a 1.5 mL sterile tube—enough volume for all samples to be amplified. 3.75 µL of this Sample Pre-Mix can then be aliquoted for each sample.
- 3 Remove these aliquots from the DNA-free hood and add 1.25 µL of cDNA to each, making a total volume of 5 µL in each aliquot.
- 4 Mix the reactions by briefly vortexing, then centrifuge.

4 Thermal Cycling

- 1 Place reaction tubes in the thermal cycler and cycle using the following table as a guide:

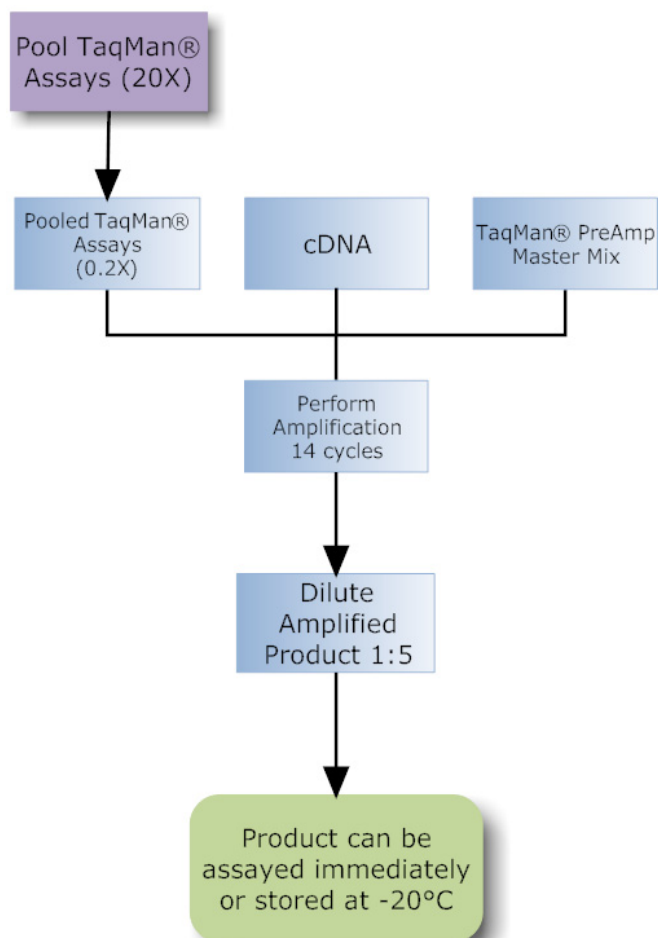
	HOLD	14 Cycles	
Temperature	95°C	95°C	60°C
Time	10 minutes	15 seconds	4 minutes

- 2 After cycling, dilute the reaction 1:5 by adding 20 μ L DNA Suspension Buffer to the final 5 μ L STA volume for a total reagent volume of 25 μ L

NOTE CYCLE NUMBER CAN BE INCREASED OR DECREASED, IF NECESSARY. PLEASE CONTACT FLUIDIGM TECHNICAL SUPPORT FOR MORE INFORMATION.

NOTE REACTIONS CAN EITHER BE ASSAYED IMMEDIATELY OR STORED AT -20°C FOR LATER USE.

Process Flow Chart



Technical Support

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