Gene Expression STA with TaqMan Master Mix and TaqMan Assays

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 microvolumes, 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 (STA) to increase target concentration.

STA uses the TaqMan® PreAmp Master Mix and TaqMan Gene Expression Assays, both from Thermo Fisher Scientific. 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.

Process Workflow

1	2	3	4	5
Pool TaqMan Assays (20X)	Mix pooled TaqMan assays (0.2X), cDNA, and TaqMan PreAmp Master Mix	Perform preamplification reactions	Dilute the amplified product 1:5	Assay the product immediately or store at −20 °C

Pool the TaqMan Gene Expression Assays

1 In a 0.5 mL microcentrifuge tube, combine equal volumes of each 20X TaqMan Gene Expression Assay, up to a total of 100 assays.

2 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:

Component	Volume (µL)
50 assays (20X)	1 (each assay)
DNA Suspension Buffer (Teknova PN T0221)	50
Total	**100**

NOTE Volume can be adjusted proportionally based on the number of samples to be amplified.

Prepare Sample Pre-Mix and Samples

NOTE Scale up the sample pre-mix and the final sample mixture appropriately for multiple samples.

1 In a DNA-free hood, prepare the sample pre-mix by combining the TaqMan PreAmp 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.

Reagent	Volume per Reaction (µL)	Volume for 48 Samples* (µL)	Volume for 96 Samples* (µL)	Volume for 192 Samples* (µL)
SAMPLE PRE-MIX				
TaqMan PreAmp Master Mix (2X) (Thermo Fisher Scientific PN 4391128)	2.5	150	300	600
Pooled assay mix (0.2X)	1.25	75	150	300
cDNA	1.25			
Final Volume	**5**			

* Includes 25% overage for ease of pipetting.

2 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.

3 Mix the reactions by briefly vortexing, then centrifuge.

Thermal-Cycle

Cycle number can be increased or decreased, if necessary. Contact Fluidigm technical support for more information.

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

Condition	Temperature	Time
Hold	95 °C	10 min
14 cycles	95 °C	15 sec
Hold	60 °C	4 min

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

NOTE Reactions can either be assayed immediately or stored at −20 °C for up to 2 weeks.

For technical support visit fluidigm.com/support.

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