Gene Expression Preamplification with Fluidigm Preamp Master Mix and TaqMan Assays

In the Biomark™ HD system, samples are loaded into individual inlets and then distributed across multiple reaction chambers in nanoliter-volume aliquots. With these small volumes, detecting the specific targets requires a minimum of 800 copies/µL in the final sample mix. For genes with lower expression levels, there are too few copies to detect adequately in cDNA samples. Preamplification is used to increase the number of copies to a detectable level for a greater number of genes.

Preamplification allows for multiplex amplification of up to 96 targets. A pool of primers is prepared from the same gene expression assays to be used for qPCR. By using the real-time qPCR assays in the preamplification reaction, only the targets of interest are amplified. A limited number of cycles is used, generally 10–14. Under these conditions of low primer concentration and a limited number of cycles, the cDNA is amplified without significant bias for the majority of genes.

Process Workflow

1	2	3	4	5
Pool TaqMan Assays	Mix pooled primers, cDNA, and 5X Preamp Master Mix	Perform preamplification reactions	Dilute the products	Assay immediately or store at −20 °C

Pool the TaqMan Gene Expression Assays

1 In a microcentrifuge tube, combine equal volumes of each 20X TaqMan® Gene Expression Assay, up to a total of 96 assays.

2 Dilute the pooled assays using Dilution Reagent (10 mM Tris-HCl, pH 8.0, 0.1 mM EDTA) so that each assay is at a final concentration of 0.2X (180 nM). The chart below provides an example using 96 assays:

Component	Volume (µL)
96 assays (20X)	2 (each assay)
Dilution Reagent (Fluidigm PN 100-8726)	8
Total	**200**

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

Prepare the Sample Pre-Mix and Samples

1 In a DNA-free hood, prepare the sample pre-mix for the reactions as shown in the following table:

Component	Vol. per Reaction (µL)	Vol. for 48 Reactions* (µL)	Vol. for 96 Reactions* (µL)	Vol. for 192 Reactions* (µL)
SAMPLE PRE-MIX				
Preamp Master Mix (Fluidigm PN 100-5744)	1.00	52.8	105.6	211.2
Pooled TaqMan assay mix (0.2X)	1.25	66.0	132.0	264.0
Water	1.50	79.2	158.4	316.8
cDNA	1.25			
Total	**5.00**			

* Includes 10% overage.

2 In a PCR plate, aliquot 3.75 µL of pre-mix for each sample.

3 Remove the plate from the DNA-free hood and add 1.25 µL of cDNA to each well containing pre-mix, making a total volume of 5 µL.

4 Mix the reactions by briefly vortexing, and then centrifuge.

Thermal-Cycle

Fourteen cycles are recommended as a starting point, but this can be decreased to 10 cycles or increased to 20 cycles, if necessary. The appropriate number of cycles should be determined empirically.

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

Condition		Temperature	Time
Hold		95 °C	2 min
14 cycles	Denaturation	95 °C	15 sec
	Annealing/extension	60 °C	4 min
Hold		4 °C	∞

2 After cycling, dilute the reaction 1:5 by adding 20 µL Dilution Reagent (Fluidigm PN 100-8726) to the final 5 µL reaction volume for a total volume of 25 µL.

NOTE Diluted reaction products can either be assayed immediately or stored at −20 °C for later use. Diluted reaction products should be stable for at least one week.

Ordering Information

Part Number	Product Name	Volume per tube (µL)
100-5580	Preamp Master Mix—1 Tube	106
100-5581	Preamp Master Mix—5 Tubes	106
100-6300	Preamp and Reverse Transcription Master Mix—1 Tube	106
100-6301	Preamp and Reverse Transcription Master Mix—5 Tubes	106

For technical support visit fluidigm.com/support.

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