

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 | 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, 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,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. .

Table 1. Sample pre-mix

| 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, 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 Pipet 3.75 μL of the sample pre-mix into each well of a 96-well PCR plate for the number of samples being preamplified.
- 3 Remove the plate from the DNA-free hood and add 1.25 μL of cDNA to each well containing sample pre-mix, making a total volume of 5 μL in each well.
- 4 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 the reaction tubes in the thermal cycler and cycle using the following table as a guide:

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

- 2 After cycling, dilute the preamplified reactions with Dilution Reagent:

| Component | Vol per Reaction (μL) |
|-----------------------------|-----------------------|
| Dilution Reagent (100-8730) | 20.0 |
| STA reaction | 5.0 |
| Total | 25.0 |

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

For technical support visit techsupport.fluidigm.com.

North America +1 650 266 6100 | Toll-free (US/CAN): 866 358 4354 | techsupport@fluidigm.com Latin America +1 650 266 6100 | techsupportlatam@fluidigm.com
Europe/Middle East/Africa/Russia +33 1 60 92 42 40 | eu.support@fluidigm.com China (excluding Hong Kong) +86 21 3255 8368 | techsupportchina@fluidigm.com
Japan +81 3 3662 2150 | techsupportjapan@fluidigm.com All other Asian countries/India/Australia +1 650 266 6100 | techsupportasia@fluidigm.com

For Research Use Only. Not for use in diagnostic procedures.

Information in this publication is subject to change without notice. **Safety data sheet information:** fluidigm.com/sds. **Patent and license information:** fluidigm.com/legal/notices. **Limited Use License to Perform Pre-amplification with Fluidigm IFCs:** A license to use Thermo Fisher Scientific's patented pre-amplification method workflows involving a Fluidigm integrated fluidic circuit (IFC) can be obtained (i) with purchase of a Fluidigm IFC from Fluidigm Corporation or (ii) by a separate license from Thermo Fisher Scientific. For licensing information, contact outlicensing@lifetech.com. **Trademarks:** Fluidigm, the Fluidigm logo, Biomark are trademarks and/or registered trademarks of Fluidigm Corporation in the United States and/or other countries. All other trademarks are the sole property of their respective owners. © 2020 Fluidigm Corporation. All rights reserved. 08/2020