TaqMan® Environmental Master Mix 2.0

Real-time PCR Master Mix for inhibited samples

- Tailored for quantitative, real-time PCR experiments
- Unrivalled sensitivity for challenging applications, low copy number target detection and multiplex PCR
- Ideal for environmental, food and other challenging samples
- Includes pathogen detection and viral/bacterial load quantification

TaqMan® Environmental Master Mix 2.0 delivers sensitive and specific detection across a broad range of template quantities, down to a single copy of target. For precise and consistent quantification, the Master Mix can detect multiple targets. Designed for analyzing environmental, food and other challenging samples, the Master Mix offers accurate pathogen detection in the presence of high levels of inhibitors. For ease of use, TaqMan Environmental Master Mix 2.0 uses universal thermal cycling conditions compatible with TaqMan Universal PCR Master Mix existing protocols.

Benefits

- Tolerance towards high levels of inhibitors
- Sensitive detection for reliable quantification of abundant and limited genetic targets
- Robust performance in multiplex PCR in which multiple targets are amplified in a single reaction
- Stable mix for high-throughput handling

Optimized Formulation for Unrivalled Performance

TaqMan Environmental Master Mix 2.0 is a convenient 2X mix for target quantification that includes:

- AmpliTaq Gold® DNA Polymerase, UP (Ultra Pure), a highly purified DNA Polymerase for improved detection of bacterial targets. This hot-start enzyme is inactive at room temperature so reactions can be set up on the benchtop. Enzyme is activated during thermal cycling.
- Passive internal reference based on proprietary ROX™ dye for precise data analysis.

Reliable Quantification of Abundant and Limited Targets

TaqMan Environmental Master Mix 2.0 provides dependable target quantification over a wide dynamic range. The amplification of a dilution series of *Salmonella* target DNA shows excellent PCR efficiency across seven orders of magnitude of template quantities using TaqMan Environmental Master Mix 2.0 (Figure 1).

Linear dynamic range across seven orders of magnitude of *Salmonella* DNA target amplification: from 2 to 2.00E+07 copies

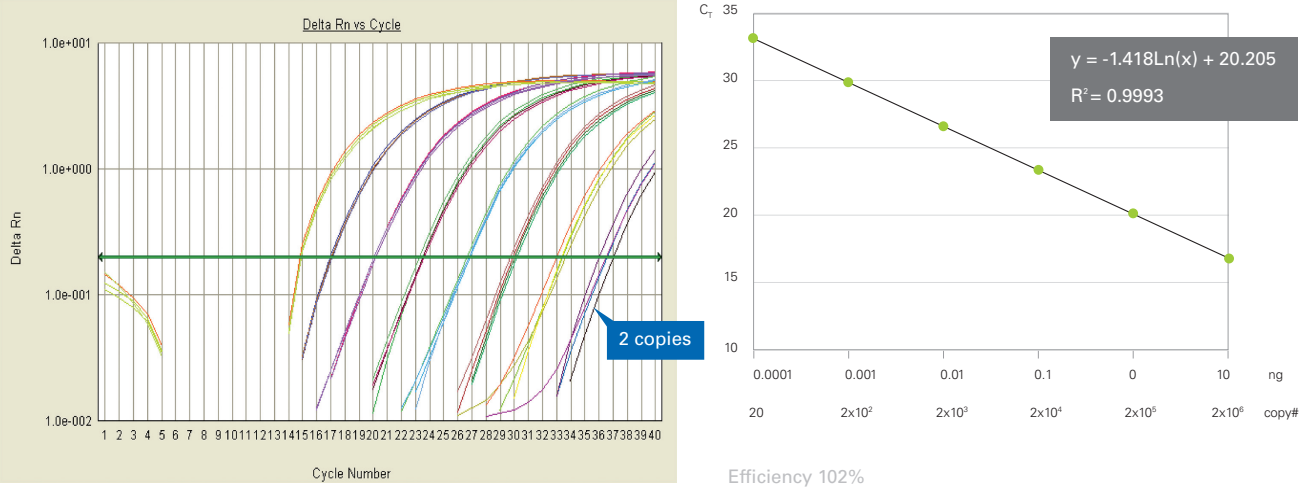
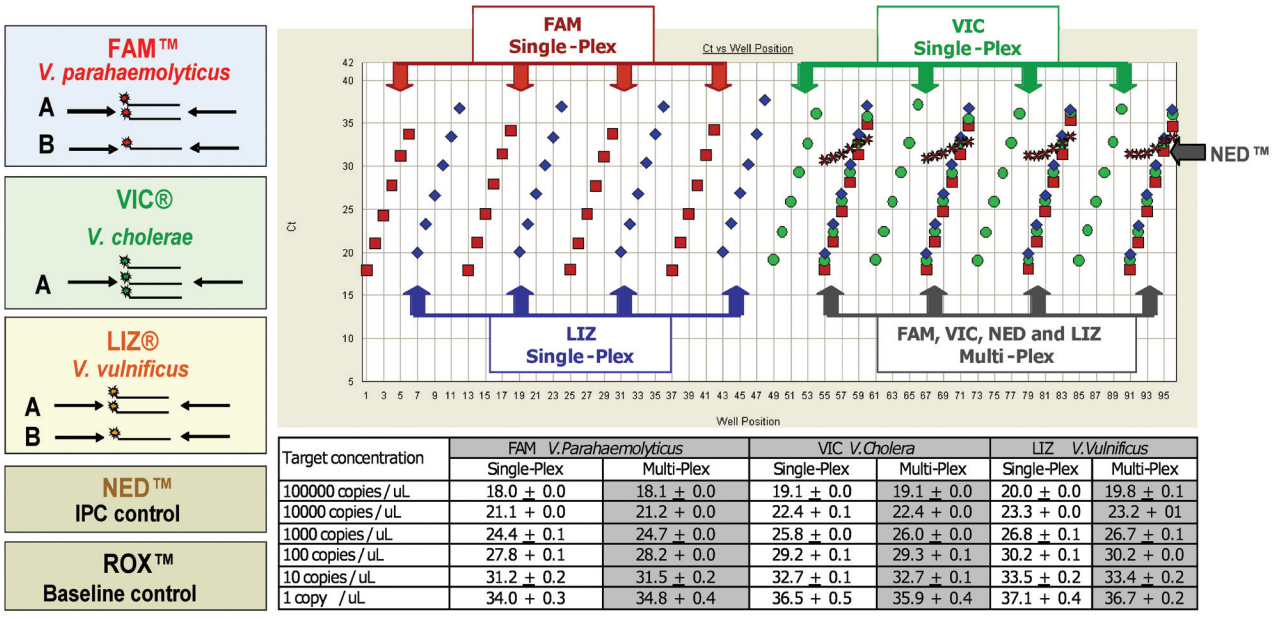


Figure 1. Amplification plot and standard curve of a *Salmonella* DNA target in four replicate reactions using TaqMan Environmental Master Mix 2.0 on Applied Biosystems 7500 Real-time PCR system.

TABLE 1. Statistical T test to evaluate detection of low amount of target

Nominal Copies	N†	Mean C_T	t-Value	p-Value	Confidence‡
2	63	36.46	4.15	<0.0001	99.9%

†Number of replicates out of 64 replicate reactions with C_T <40. Due to sampling error, the Poisson distribution predicts that some samples with very few targets per reaction contain zero copies of target.
‡ Confidence is the probability that the mean C_T of samples with fewer input copies of target is greater than the mean C_T of samples that contain more input copies of target.



Target concentration	FAM *V.Parahaemolyticus*		VIC *V.Cholera*		LIZ *V.Vulnificus*	
	Single-Plex	Multi-Plex	Single-Plex	Multi-Plex	Single-Plex	Multi-Plex
100000 copies / uL	18.0 ± 0.0	18.1 ± 0.0	19.1 ± 0.0	19.1 ± 0.0	20.0 ± 0.0	19.8 ± 0.1
10000 copies / uL	21.1 + 0.0	21.2 + 0.0	22.4 + 0.1	22.4 + 0.0	23.3 + 0.0	23.2 + 01
1000 copies / uL	24.4 ± 0.1	24.7 ± 0.0	25.8 ± 0.0	26.0 ± 0.0	26.8 ± 0.1	26.7 ± 0.1
100 copies / uL	27.8 + 0.1	28.2 + 0.0	29.2 + 0.1	29.3 + 0.1	30.2 + 0.1	30.2 + 0.0
10 copies / uL	31.2 ± 0.2	31.5 ± 0.2	32.7 ± 0.1	32.7 ± 0.1	33.5 ± 0.2	33.4 ± 0.2
1 copy / uL	34.0 + 0.3	34.8 + 0.4	36.5 + 0.5	35.9 + 0.4	37.1 + 0.4	36.7 + 0.2

Figure 2. Multiplex *Vibrio* detection assay configuration and real-time PCR detection using TaqMan Environmental Master Mix 2.0. C_T values plotted vs. well position for *V.parahaemolyticus* (FAM™, red) *V.cholera* (VIC®, green) and *V.vulnificus* (LIZ®, blue) detection demonstrate similar quantitation of single targets by a corresponding single-dye assay and mixed targets by the four-dye *Vibrio* detection assay on Applied Biosystems 7500 Real-time PCR system.

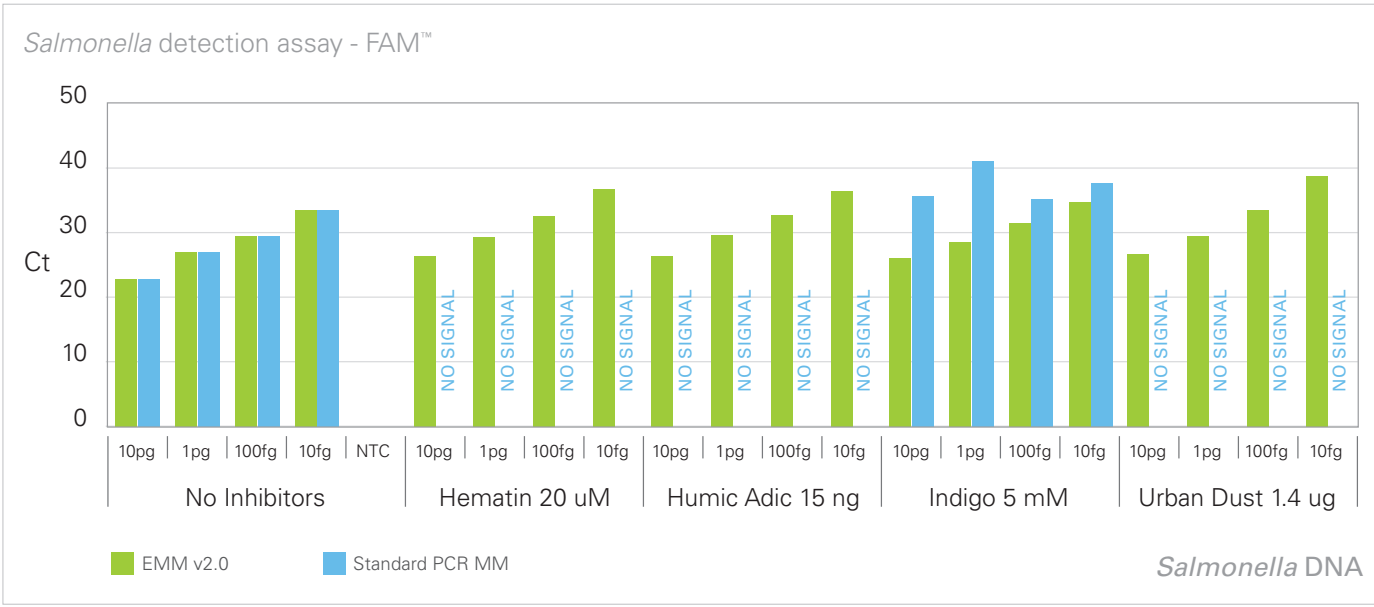


Figure 3. The C_T values for amplification of *Salmonella* DNA target performed in dilution series with and without various inhibitors: Hematin 20 µM, Humic acid 15 ng per reaction, Indigo 5 mM and Urban dust 1.5 µg per reaction. TaqMan Environmental Master Mix 2.0 was compared to a commonly used PCR Master Mix using Applied Biosystems 7500 Real-time PCR system.

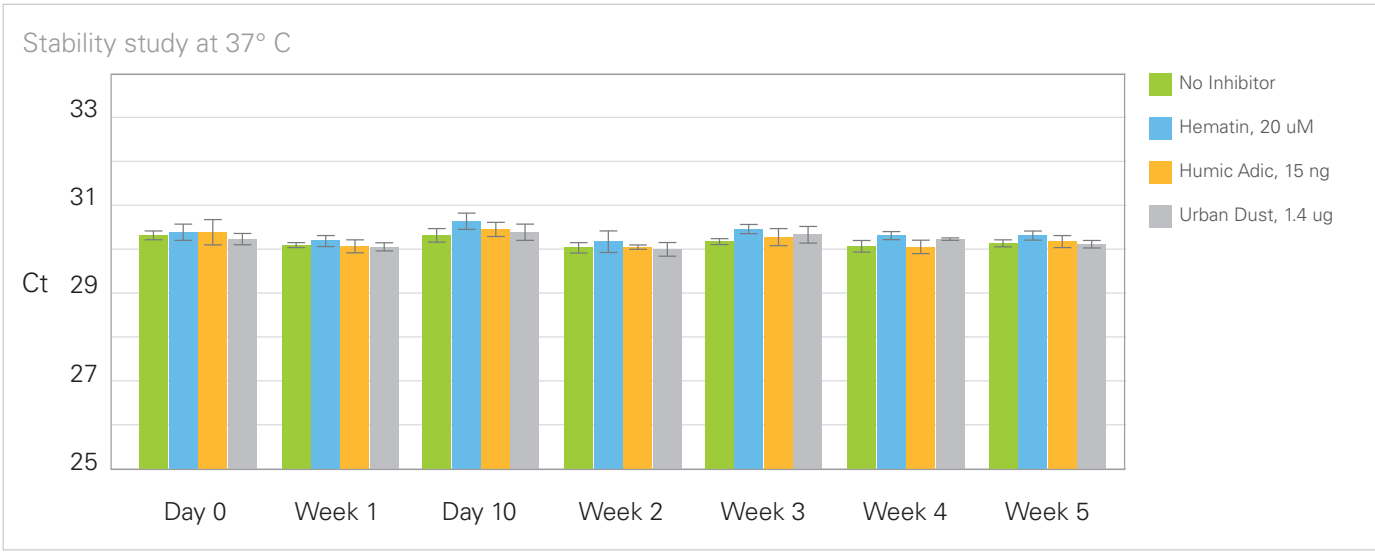


Figure 4. The C_T values for amplification of *Salmonella* DNA target performed with and without various inhibitors: Hematin 20 µM, Humic acid 15 ng per reaction, Indigo 5 mM and Urban dust 1.5 µg per reaction. TaqMan Environmental Master Mix 2.0 was stored at 37°C . PCR was performed immediately (Day 0), or following storage for 5 weeks, using Applied Biosystems 7500 Real-time PCR system.

The sensitivity of TaqMan Environmental Master Mix 2.0 was validated using quantified *Salmonella* DNA target. Since significant sampling error occurs when measuring low quantities of target, proper evaluation requires statistical analysis of multiple replicates. Figure 1 shows the expected quantity of target and corresponding mean C_T values. Statistical analysis indicates high confidence of sample quantification based on a T-test (Table 1), consistent with 1-2 copy detection of target. TaqMan Environmental Master Mix 2.0 enables detection of low quantities of target.

Reliable Multiplex Detection of Mixed Target DNAs

TaqMan Environmental Master Mix 2.0 provides dependable quantification of mixed targets over a wide dynamic range. The amplification of a dilution series of *Vibrio* species target DNAs (*V.parahaemolyticus*, *V.cholera* and *V.vulnificus*) shows excellent PCR efficiency across five orders of magnitude of template quantities using TaqMan Environmental Master Mix 2.0 (Figure 2). Testing of a single target by a single-dye assay (FAM™, VIC® or LIZ®) demonstrates similar detection of the same target in mixed sample (three DNA targets) by the four-dye *Vibrio* detection assay.

Enhanced Performance in the Presence of Inhibitors

TaqMan Environmental Master Mix 2.0 provides reliable detection of DNA targets in the presence of common PCR inhibitors. Amplification of a dilution series of Salmonella target DNA shows excellent PCR efficiency of the template with and without high levels of inhibitors (Hematin 20 µM, Humic acid 15 ng per reaction, Indigo 5 mM and Urban dust 1.5 µg per reaction) while a commonly used TaqMan Master Mix shows significant inhibition in the presence of all inhibitors and across target DNA concentrations tested (Figure 3).

Stable Mix for Environmental Testing

Extended stability of the TaqMan Environmental Master Mix 2.0 allows flexibility to process numerous samples at room temperature. The stability of TaqMan Environmental Master Mix 2.0 was demonstrated using 100 copies of *Salmonella* DNA amplified with and without inhibitors. The stability study was performed at 37°C over 5 weeks. All 28 tests show equivalent amplification for the time points tested (Figure 4). Even after 5 weeks, the excellent stability of TaqMan Environmental Master Mix 2.0 provides accurate and consistent results.

TABLE 2. Instruments, Assays, and Reagents Compatibility Data (standard thermal cycling mode)

StepOne™ Real-Time PCR System	Yes
Applied Biosystems 7300 Real-Time PCR System	Yes
Applied Biosystems 7500 Real-Time PCR System	Yes
Applied Biosystems 7500 Fast Real-Time PCR System	Yes
Applied Biosystems 7900HT Fast Real-Time PCR System	Yes
ABI PRISM® 7000 Sequence Detection System	Yes
ABI PRISM® 7900HT Sequence Detection System	Yes
TaqMan® Reverse Transcription Reagents	Yes
High Capacity cDNA Reverse Transcription Kit	Yes

ORDERING INFORMATION

Description	Quantity	Reactions*	P/N
TaqMan® Environmental Master Mix 2.0, 1-Pack	5 mL bottle	200	4396838
TaqMan® Environmental Master Mix 2.0, 2-Pack	10 mL bottle	400	4398021
TaqMan® Environmental Master Mix 2.0, 4-Pack	20 mL bottle	800	4398044

**assumes 50 µL reaction volume*

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