Datasheet

Bio-Star 2X Universal-flex Multiplex qPCR Master for probes with UDG/dUTP

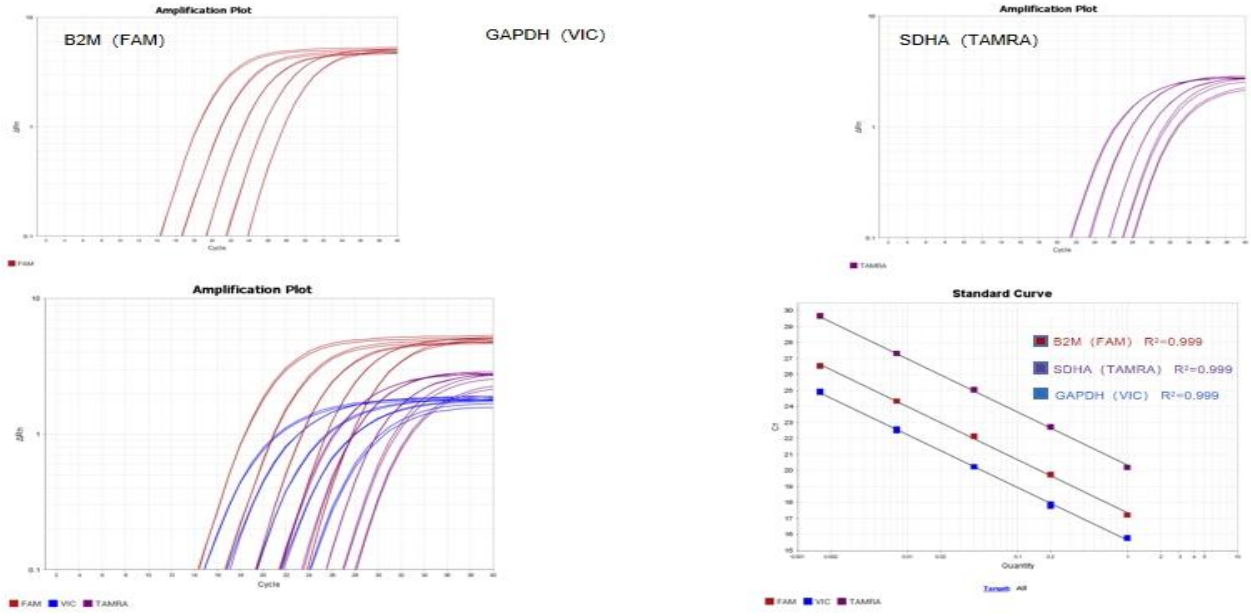
Description

The Bio-Star 2X Universal-flex Multiplex qPCR Master Mix is a reaction mix optimized for real-time qPCR detection and quantitation of target DNA up to four sequences using hydrolysis probes. It contains Hot Start Taq DNA polymerase and all components like dNTPs (**dUTP** instead dTTP), UDG, $MgCL_2$, reaction buffer additives and stabilizers, optimized for Probe qPCR to ensures:

- a perfect amplification curve
- no need to adjust ROX concentration
- accurate quantification of target genes
- increased PCR specificity and sensitivity
- good repeatability and high reliability
- over a broad dynamic range
- UDG/dUTP prevent residual contamination

Only templates, primers, probes and Nuclease-free Water need to be added for use. It also features a **unique passive reference dye** that is compatible across a variety of instrument platforms and a non-fluorescent **blue visible dye** to monitor reaction setup. This dye does not spectrally overlap fluorophores commonly used for qPCR and will not interfere with real-time detection.

Storage and transportation: at -20 °C. Shipping with blue ice
Storage terms: up to 18 months



Picture: Expression of human B2M, GAPDH and SDHA genes respectively.
In the 20μL amplification system, the templates were 300ng, 60ng, 12ng, 2.4ng, 0.48ng (5x dilution) of human cDNA. The primer concentration was 0.2μM.
Two duplicate wells were made for each concentration gradient sample.
The experimental results as shown in the picture above, the lower left picture is the result of superposition of three genes, and the lower right picture is the standard song.

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Universal qPCR Mastermix: Amplification protocol

1. Defrost the reaction mixture and stir thoroughly.
2. Add the following components into the thin-wall PCR tubes considering the final volume of a reaction mixture equal to 20 µl:

Component	Volume	Final concentration
2x Mastermix with UDG	10 µl	1x
Each Forward Primer 10µM *1	variable	0,2 µM
Each Reverse Primer 10 µM *1	variable	0,2 µM
Probe 10 µM *1	variable	
DNA Template *2	variable	10 pg - 1 µg
Sterile Water	up to 20 µl	

3. Gently vortex and remove droplets by centrifugation.
4. Perform PCR

***1**: Usually, a good amplification effect can be obtained with the final concentration of 0.2 µM. When the reaction performance is poor, the primer concentration can be adjusted in the range of 0.2-1.0 µM.
***2**: The amount of template added varies depending on the number of copies of the target gene, and the appropriate amount of template addition is studied by gradient dilution. The best addition amount of template DNA in the 20 µl reaction system was less than 100 ng.

Universal qPCR Mastermix Cycler program

Step	Temp. °C	Incubation time	Number of Cycles
UDG Incubation	50	2 min	1
Preliminary denaturation	95	0,5 min	1
Denaturation	95	15 sec	30-40
Annealing	55-65	10 sec	30-40
Elongation	72	30 sec	30-40 ***1**
Melting curve (recommended)	1		1

as an alternative:

Step	Temp. °C	Incubation time	Number of Cycles
UDG Incubation	50	2 min	1
Preliminary denaturation	95	0,5-2 min	1
Denaturation	95	15 sec	30-40
Annealing / Extension	60	30 sec	30-40 ***1**

Elongation	72	30 sec	30-40
Melting curve (recommended)	1		1

***1**: If amplification specificity needs to be improved, two-step procedure or annealing temperature can be used; To improve the amplification efficiency, a three-step procedure or extension time can be used.

Compatible instruments / Cycler List

ABI: 5700, 7000, 7300, 7700, 7900, 7900HT, 7900 HT Fast, StepOne™, StepOne Plus™, 7500/7500 Fast, ViiA 7™,;
Analytik Jena: qTOWER series;
qTOWER: LineGene series
Stratagene: Mx3000P®, 3005P™, 4000™;
Bio-Rad: CFX96™, CFX384™, iCycler iQ™, iQ5™, MyiQ™, MiniOpticon™, Opticon®, Opticon 2, Chromo4™;
Eppendorf: Realplex 2s, Mastercycler® ep, Realplex;
Illumina: Eco QPCR;
Cepheid: SmartCycler®; QuantStudio™ series, PikoRealTM Cycler
Qiagen Corbett: Rotor-Gene® series;
Roche: LightCycler™ series;
Takara: Thermal Cycler Dice series;

Please note:
1. After thawing, please gently mix up and down, do not vortex, avoid bubbles, mix well before use.
2. When preparing the reaction solution, please place the reagent on the ice.
3. The product contains fluorescent dyes, so strong light should be avoided when preparing qPCR reaction solution.
4. New disposable tips should be used for preparation of reaction mixes to avoid cross contamination.
5. Avoid freeze-thawing cycles of the Master Mix, and try to use it up within a month after thawing.

Cat.-no	Description	Amount
M150	**Bio-Star 2X Universal-flex probe Multiplex qPCR Master (Luna3)**	2 x 1 ml
M150L	**Bio-Star 2X Universal-flex probe Multiplex qPCR Master (Luna3)**	10 x 1 ml