

## Bio-Star 2X Universal-flex probe qPCR Master

### Description

The Bio-Star 2X Universal-flex probe qPCR Master Mix is a reaction mix optimized for real-time qPCR detection and quantitation of target DNA sequences using hydrolysis probes. It contains Hot Start Taq DNA polymerase and all components like dNTPs, MgCL<sub>2</sub>, reaction buffer additives and stabilizers, optimized for Probe qPCR to ensures:

- a perfect amplification curve
- accurate quantification of target genes
- increased PCR specificity and sensitivity
- good repeatability and high reliability
- over a broad dynamic range.

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, **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

### **Universal qPCR Mastermix (two fold concentrated) 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 50 µl:

Component	Volume	Final concentration
2x Mastermix	25 µl	1x
Forward Primer *1	variable	0,2 µM
Reverse Primer *1	variable	0,2 µM
DNA Template *2	variable	10 pg - 1 µg
Sterile Water	up to 50 µl	

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

\*1: a: 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: b: 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 Cyclers program

Step	Temp. °C	Incubation time	Number of Cycles
Preliminary denaturation	95	0,5-2 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
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, PikoReal™ Cycler

**Qiagen Corbett:** Rotor-Gene® series;

**Roche:** LightCycler™ series;

**Takara:** Thermal Cycler Dice series;

Cat.-no	Description	Amount
M130	Bio-Star 2X Universal-flex probe qPCR Master	2 x 1 ml
M130L	Bio-Star 2X Universal-flex probe qPCR Master	10 x 1 ml