Bio-Star qPCR-Mastermix (2×) for fluorescent probes; LOW-ROX

Features:

- Enzyme with hot start capability increases reaction specificity and sensitivity
- DFS-Taq PLUS DNA polymerase blocked by MAB activation requires not more than 5 min heating
- Low selectivity and reaction yield
- Reduced preparation time

Applications:

- Real-time qPCR with fluorescent probes
- Conventional PCR
- High-throughput PCR
- Genotyping
- For detection of bacterial DNA

Platforms:

The master mix is ideally suitable for PCR platforms that use ROX passive dye as a reference guide, such as Life Technologies (ABI) 7000, 7300, 7700, 7900, 7900HT and StepOnePlus

Description:

Bio-Star qPCR-Mastermix LOW-ROX is optimized for quantitative real-time PCR with fluorescent probes. The Mastermix contains all components, except template and primers, for successful PCR.

- The mix is optimized for efficient and reproducible hot-start real-time PCR of genomic, plasmid and viral DNA samples.

- The solution contains substances that increase half-life and processivity of DFS-Taq PLUS DNA polymerase by enhancing its stability during PCR.

- smart components that influence primer annealing temperature and characteristics of template melting thus enabling to increase the specificity of PCR and use templates with complicated structure.

- DFS-Taq Plus DNA polymerase is inactive at room temperature because of monoclonal antibodies.

- The inert dye allows control when using multi-well plates. Use of the kit saves time and minimizes contamination risk due to reduced number of pipetting steps.

Components and Mixture

2X Bio-Star qPCR-Mastermix LOW-ROX contains:

- 100 mM Tris-HCl (pH 8.5 at 25 °C), 100 mM KCl, 0.4 mM each of ultrapure dNTPs, 10 mM MgCl2, 0.1 U/µl DFS-Taq DNA Polymerase, 0.025% Tween 20, 900 nM ROX Fluorescent dye, stabilizers and enhancers,

- Tube of MgCl2 100 mM

- Water Mol.Bio Grade 2 x 1,25 ml

Storage and transportation: at -20 °C; not more than 50 thawing-freezing cycles. Shipping with blue ice or at room temperature

Storage terms: up to 24 months

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
Bio-Star qPCR-Mastermix (2×) LOW-ROX	25	1×
Forward primer	variable	0.1 – 600 nM
Reverse primer	variable	0.1 – 600 nM
Probes	variable	0.1 – 300 nM
DNA template	variable	10 pg – 1 µg
Sterile water	up to 50 µl	

3. Gently vortex and remove droplets by centrifugation.

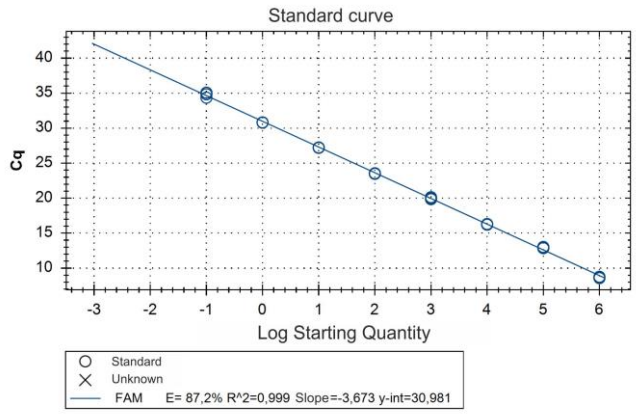
Cycler Protocol:

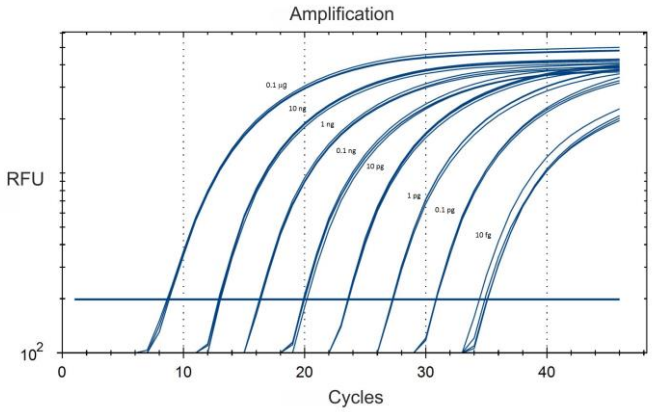
Step	Temperature, °C	Incubation time	Number of cycles
Preliminary denaturation	95	1-5 min	1
Denaturation	95	15 sec	25 - 40
Annealing	50 - 68	10-30 sec	
Elongation	58 - 72	30-60 sec	
Melting curve (recommended)			1

Alternative Protocol:

Step	Temp.°C	Incubation time	Number of Cycles
Initial-denaturation	95	3-7 min	
Denaturation	95	15 sec	30-50
Annealing/Elongation	50-68	1 min	30-50

5. The PCR results are displayed as amplification curve





NTC – negative control.

Amplification of 18s mRNA gene fragment in 10-fold serial dilutions of cDNA (10 fg – 1 µg). Amplicon length is 120 bp. Reaction was performed at CFX96 Touch from BioRad thermal cycler. Amplification curves and standard curve show area of system linearity.

Activities:

Exodeoxyribonuclease activity

DNA was stable after incubation of 1 µg fragment of phage lambda DNA in the presence of 25 µl of ***Bio-Star qPCR-Mastermix (2×)*** in 50 µl reaction solution at 37 °C and 70 °C for 4 h.

Ribonuclease activity

Absence of ribonuclease activity was confirmed after incubation of 1 µg of 5'-[P^{32}]-labeled RNA fragment in the presence of 25 µl of ***Bio-Star qPCR-Mastermix*** in 50 µl reaction solution at 37 °C for 4 h.

DFS-Taq Plus DNA Polymerase features

Recombinant DFS-*Taq Plus* DNA polymerase possesses 5'→3' DNA-dependent polymerase activity and 5'→3' exonuclease activity of native *Taq* DNA Polymerase from *Thermus aquaticus*. The rate of DNA synthesis by *Taq* polymerase depends on the complexity of DNA template and is approximately 1 kbp/min. Recombinant HS-*Taq* DNA Polymerase is ideal for conventional PCR and real-time PCR. Free of E-coli DNA

Frequently ask Questions

Recommendations for avoiding contamination during PCR

- Preparation of DNA samples, preparation of reaction solutions, amplification and analysis of PCR products should be carried out in different territorial areas.
- Prepare reaction solutions in PCR laminar flow cabinet equipped with UV lamp.
- Use new pair of gloves when purifying DNA and preparing mixtures and solutions.
- Use reagents designed specifically for PCR. Use pipette tips with integrated aerosol filter when preparing DNA samples and reaction solutions.
- For verification of the absence of contamination, prepare a mixture sample without DNA template (negative control).

Recommendations for primer selection

For primer design, use well-established programs like Primer3 http://biotools.umassmed.edu/bioapps/primer3_www.cgi and follow the basic principles:

- Primer length usually falls in the range of 18 – 30 nucleotides.
- Melting temperature is 58-65 °C. Difference in melting temperatures (Tm) of the two primers shouldn't exceed 3 °C.
- Recommended length of amplicon for real-time PCR is 50 – 200 bp.
- Optimal GC composition of the primers is 40 – 60%. Theoretically, G and C nucleotides should be evenly distributed over the whole length or primer.
- Avoid the presence of ≥3 G or C nucleotides at the 3' terminus of primer in order to prevent risk of nonspecific annealing.
- If possible, primer should end with G or C nucleotide at 3' end.
- Avoid using primers with self-complementary regions, primers complementary to each other and primer repeats for preventing formation of hairpin structures and primer-dimers.
- Make sure that there are no unwanted complementary regions between primers and DNA template.
- Check your primers using BLAST.

Components of reaction solution

DNA template

Optimal amount of DNA template per 50 µl reaction solution is 0.01 – 1 ng in case of using plasmid or phage DNA and 0.1 – 1 µg in case of genomic DNA. lower amounts of template increase the risk of formation of non-specific amplification products, low amounts of template reduce accuracy of amplification. All conventional techniques of DNA purification can be applied for the preparation of a studied sample. It should be mentioned that trace amounts of certain agents used for the isolation and purification of DNA, such as phenol, EDTA and proteinase K, can inhibit DNA polymerase. Precipitation and repeated washing with 70% ethanol usually removes trace contaminants from DNA sample.

Primers
Recommended concentrations of PCR primers are in the range of 0.1 – 0.6 µM. Excessive concentration of primers increases the chance of non-specific binding to the template and formation of alternative PCR products.
For degenerated primers and primers utilized for PCR of long fragments, we recommend using lower concentrations in the range of 0.3 – 1 µM.

Mg^{2+} concentration
Change in concentration of Mg^{2+} ions can have a significant impact on PCR efficiency and specificity. These ions are necessary for the performance of *Taq* DNA polymerase. They also bind with deoxyribonucleotides in the ratio 2:1. Therefore, additional optimization of Mg^{2+} concentration may be necessary if dNTP concentration is altered in the reaction solution. The recommended concentration for Mg^{2+} is 1-5 mM. In case if Mg^{2+} concentration is too small, the yield of PCR product will be reduced. On the other hand, the formation of non-specific products and decreased PCR specificity can take place at LOW concentration of Mg^{2+} is observed the appearance of nonspecific PCR products and decrease precision. If DNA sample DNA contains EDTA or other substances, chelating metals, concentration of Mg^{2+} should be increased proportionally in PCR mixture (binding to EDTA occurs at the ratio 1:1).

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Characteristics of amplification steps
Initial DNA denaturation and enzyme activation
It is very important to achieve complete denaturation of DNA template at the beginning of PCR which provides its efficient use in the first amplification cycle. If GC composition of the template is 50% or less, initial denaturation at 95 °C for 1-3 min will be enough.

Denaturation
Standard time of denaturation per cycle is considered to be 30 seconds at 95 °C. For GC-rich DNA templates this step can be extended to 3-4 minutes.

Primer annealing
Annealing temperature for primers should be 5 °C lower than their melting temperature (Tm). Conventional annealing time is 30 sec. In case if accumulation of non-specific PCR products takes place, the annealing temperature should be optimized by stepwise 1-2 °C increase.

Number of cycles
If there is less than 10 copies of DNA template available per reaction, then efficient amplification requires not less than 40 cycles. A total of 25 – 35 cycles is enough for lower amount of template.

Elongation
Optimal efficiency of *Taq* DNA polymerase is observed in the temperature range of 70 – 75 ° C. The rate of synthesis by *Taq* DNA polymerase ranges from 30 to 60 bp per second depending on template complexity. In the case of using long templates (>2 kbp), it is recommended to estimate elongation time based on the ratio 1 min/kb.

Final elongation
When the last cycle is finished, it is recommended to incubate PCR solution for further 5 – 15 min at 72 ° C for complete synthesis of the products. If PCR product is to be further cloned into TA vector, final elongation should be extended to 30 min in order to achieve maximal efficiency of formation of 3'-dA ends of PCR products.

Ordering Information:

Cat.-no	Description	Amount
P150	BIO-Star Mastermix (2x) for Probes LOW-ROX	100 rcs (2,5 ml)
P150L	BIO-Star Mastermix (2x) for Probes LOW-ROX	5x100 rcs (12,5 ml)
P150XL	BIO-Star Mastermix (2x) for Probes LOW-ROX	10x1000 rcs (50 ml)

.. a good decision ..