Biozym Blue Probe qPCR Mix Separate ROX

Product information

Store all kit components at -20°C with minimal exposure to light.
The kit may be stored at 4°C for short term use (up to 1 month). Multiple freeze/thaw cycles (up to 30) are possible with no loss of activity.

Component	331456X 10 rxn	331456S 100 rxn	331456L 500 rxn	331456XL 2500 rxn
Blue Probe qPCR 2x Mix	1 x 0.2 ml	1 x 1.0 ml	5 x 1.0 ml	25 x 1.0 ml
50 µM ROX Additive	1 x 0.05 ml	1 x 0.2 ml	5 x 0.2 ml	25 x 0.2 ml

For research use only.

1. Introduction

Biozym Blue Probe qPCR Mix is designed for quantitative, real-time analysis of DNA and cDNA samples. Used polymerase technology along with progressive buffer chemistry enable fast protocols, enhanced specificity and reaction efficiency.

Biozym Blue Probe Mixes are designed for use with probe technologies including TaqMan®, Scorpions® and molecular beacons.

The mix contains a non-reactive blue that helps keeping track of pipetting the master mix into the wells. It supports to identify if a well is empty or already loaded with the blue master mix. The dye does not interfere with DNA polymerase reaction but will influence the intensity of some fluorescent dyes.

The used hot-start technology inhibits DNA polymerase activity at ambient temperature, thus preventing formation of primer-dimers to improve reaction sensitivity and specificity.

The reaction chemistry is applicable to most qPCR instruments. ROX Additive is added separately if the instrument requires such as passive reference dye.

2. Notes

- Master Mix contains a non-reactive blue dye for keeping track of pipetting.
- ROX passive reference dye is included separately (see 5.4).

3. Technical support

For technical support please contact support@biozym.com

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4. Reaction conditions for qPCR

Carefully mix and centrifuge the tubes before opening to ensure homogeneity and improve recovery.

Table 1. Pipetting instructions

Component	20 µl reaction	Final concentration	Notes
2x qPCR Blue Probe Mix	10 µl	1x	
Forward primer (10 µM)	0.8 µl	400 nM	See 5.2.
Reverse primer (10 µM)	0.8 µl	400 nM	
Probe (10 µM)	0.4 µl	200 nM	See 5.3.
Template DNA cDNA gDNA	 <100 ng <1 µg	Variable	
PCR grade water	Up to 20 µl reaction volume		

Table 2. Cycling instructions

Cycles	Temperature	Time	Notes
1	95°C	2 min	Initial denaturation and enzyme activation, 2 min for cDNA, 3 min for gDNA
40	95°C 60 to 65°C	5 seconds 20 to 30 seconds	Denaturation Annealing/Extension, do not exceed 30 seconds or temperatures below 60°C

5. Notes about reaction components and cycling conditions

5.1. Biozym Blue Probe qPCR Mix

The 2x mix contains a hot-start DNA polymerase, buffer, dNTPs and a blue dye that helps keeping track of pipetting the master mix into the wells.

5.2. Primers

For best efficiency of the reaction the amplicon length should be between 80 and 200 bp. Short amplicons enable fast cycling conditions. Primers should have a calculated melting temperature of around 60°C, using default Primer 3 settings (http://frodo.wi.mit.edu/primer3/). The final primer concentration in the reaction should be 0.4 µM (0.2 – 0.4 µM).

5.3. Probe intensity

The blue dye in the Blue Probe Mix will absorb light at excitation and emission wavelengths and lower the fluorescent intensity from probes. Despite this circumstance a probe concentration of 200 nM (see table 1.) shows significant detection on all instruments tested.

Fluorophore	Ex / Em [nm]	Signal loss
FAM	494 / 518	12%
HEX	535 / 556	55%
Texas Red	595 /615	88%
Cy5	675 / 694	82%

5.4. ROX passive reference dye

Instrument compatibility

Different real-time PCR instruments may require different levels of ROX passive reference.

Addition of ROX additive

The 50 µM ROX Additive supplied is formulated to be added directly to the 1 ml tube of Biozym master mix supplied. Once the ROX is added, the reagent may be directly used or stored at -20°C. Please follow the below table to add the correct amount of ROX for your instrument. Mix carefully after ROX addition.

Table 3. ROX for Hi-ROX instruments

Reagent	Hi-ROX instruments	Final concentration	Reaction concentration
2x Blue Probe 2x Mix	1.0 ml (0.2 ml)	2x	1x
50 µM ROX Additive	35.0 µl (7 µl)	1.75 µM	875 nM

Table 4. ROX for Lo-ROX instruments

Reagent	Lo-ROX instruments	Final concentration	Reaction concentration
2x Blue Probe 2x Mix	1.0 ml (0.2 ml)	2x	1x
50 µM ROX Additive	4.0 µl (0.8 µl)	200 nM	100 nM