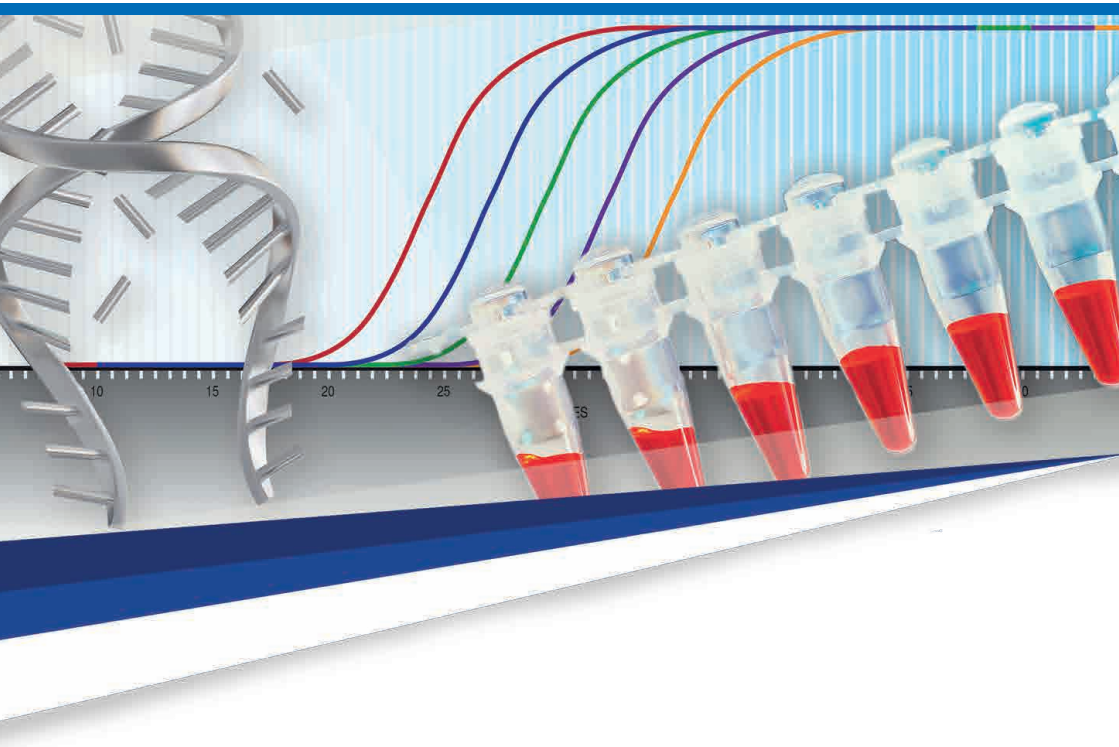
MP Biomedicals

Instruction Manual



qPCR & Go Probe No-Rox Kit

Storage:
-20°C

Cat. No:	Reactions:	Size:
11EBI06002	20	200 µL
11EBI06050	500	5 x 1 ml
11EBI06200	2000	4 x 5 ml

Protocol Revision:
119F0318-1

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1. Description

The qPCR & Go Probe No-ROX Kit has been developed for fast, highly reproducible real-time PCR and has been validated on commonly used real-time PCR instruments. The kit has been formulated for use with probe-detection technology, including TaqMan®, Scorpions® and molecular beacon probes.

A combination of the latest advances in buffer chemistry and PCR enhancers, together with a hot-start DNA polymerase, ensures that the qPCR & Go Probe Kit delivers fast, highly-specific and ultra-sensitive real-time PCR.

2. Ordering information

Reagent	Cat. No.	20 reactions	500 reactions	2000 reactions
qPCR & Go Probe No-ROX mix (2x)	11EBI06002	200 µl	-	-
	11EBI06050	-	5 x 1 ml	-
	11EBI06200	-	-	4 x 5 ml

3. Storage and Stability

The qPCR & Go Probe No-ROX Kit is shipped on dry/blue ice. All kit components should be stored at -20°C upon receipt. Excessive freeze/thawing is not recommended.

4. Expiry

When stored under the recommended conditions and handled correctly, full activity of the kit is retained until the expiry date on the outer box label.

5. Quality control

The qPCR & Go Probe No-ROX Kit and its components are extensively tested for activity, processivity, efficiency, heat activation, sensitivity, absence of nuclease contamination and absence of nucleic acid contamination.

6. Safety Precautions

Please refer to the material safety data sheet for further information.
Available on **www.mpbio.com/msds.php**

7. Note

This reagent has been manufactured under 13485 Quality Management System and is suitable for further manufacturing use as an IVD component.

8. Instrument compatibility

The qPCR & Go Probe No-ROX Kit is compatible with real-time PCR instruments that do not need a passive reference signal for normalization of the data. The qPCR & Go Probe No-ROX Kit has been optimized for use on the real-time PCR instruments listed in the following compatibility table.

Manufacturer	Model
Bio-Rad	iCycler®, iQ™5, MyiQ™, Opticon™, Opticon2™, MiniOpticon, Chromo4™, CFX96, CFX384
Cepheid	SmartCycler™
Qiagen	Rotor-Gene™ 3000 & 6000
Eppendorf	Mastercycler® ep realplex
Roche	LightCycler® 480
Techne	Quantica®
BMS	Mic
Takara	Thermal Cycler Dice® (TP800)

9. General considerations

To help prevent any carry-over DNA contamination, we recommend that separate areas are maintained for reaction set- up, PCR amplification and any post-PCR gel analysis. It is essential that any tubes containing amplified PCR product are not opened in the PCR set-up area.

Primers and probe: These guidelines refer to the design and set-up of TaqMan probe-based PCR. Please refer to the relevant literature when using other probe types. The specific amplification, yield and overall efficiency of any real-time PCR can be critically affected by the sequence and concentration of the probes and primers, as well as by the amplicon length.

We strongly recommend taking the following points into consideration when designing and running your real-time PCR:

- Use primer-design software, such as Primer3 *(http://frodo.wi.mit.edu/primer3/)* or visual OMP™ *(http://dnasoftware.com/)*. Primers should have a melting temperature (Tm) of approximately 60°C; the Tm of the probe should be approximately 10°C higher than that of the primers.

- Optimal amplicon length should be 80-200 bp, and should not exceed 300 bp.

- Final primer concentration of 400 nM is suitable for most Probe-based reactions, however to determine the optimal concentration we recommend titrating in the range 0.2-1 µM. The forward and reverse primers concentration should be equimolar.

- A final probe concentration of 100 nM is suitable for most applications; we recommend that the final probe concentration is at least two-fold lower than the primer concentration.

Note: In multiplex real-time PCR probe concentrations over 100nM can result in cross-channel fluorescence.

Template: It is important that the DNA template is suitable for use in PCR in terms of purity and concentration. In addition, the template must be devoid of any contaminating PCR inhibitors (e.g. EDTA). The recommended amount of template for PCR is dependent upon the type of DNA used. The following points should be considered when using genomic DNA and cDNA templates:

- **Genomic DNA:** use up to 1 µg of complex (e.g. eukaryotic) genomic DNA in a single PCR.

- **cDNA:** the optimal amount of cDNA to use in a single PCR is dependent upon the copy number of the target gene; we suggest using 100 ng cDNA per reaction, however it may be necessary to vary this amount; to perform a two-step RT-PCR, we recommend using the cDNA & Go Synthesis Kit (Cat N°: 11EBI00005) for reverse transcription of the purified RNA.

$MgCl_2$: The qPCR & Go Probe mix contains an optimized concentration of $MgCl_2$, it is not necessary to supplement the mix further.

PCR controls: It is important to detect the presence of contaminating DNA that may affect the reliability of the data. Always include a no-template control (NTC) reaction, replacing the template with PCR-grade water. When performing a two-step RT-PCR, set up a no-RT control as well as an NTC for the PCR.

10. Procedure

Reaction mix composition: Prepare a PCR mastermix. The volumes given below are based on a standard 20 µL final reaction mix and can be scaled accordingly.

Reagent	Volume	Final Concentration
2x qPCR & Go Probe No-ROX Mix	10 µL	1x
10 µM Forward Primer	0.8 µL	400 nM
10 µM Reverse Primer	0.8 µL	400 nM
10 µM Probe	0.2 µL	100 nM
Template	up to 8.2 µL	-
H_2O	As required	-

Sensitivity testing and C_t values: When comparing qPCR & Go Probe mix with a mix from another supplier we strongly recommend amplifying from a 10-fold template dilution series. Loss of detection at low template concentration is the only direct measurement of sensitivity. An early C_t value is not an indication of good sensitivity, but rather an indication of speed.

Suggested thermal cycling conditions

The real-time PCR conditions, in the table below, are suitable for the qPCR & Go Probe No-ROX Kit with the amplicons of up to 200 bp. These cycling parameters have been optimized on a number of platforms, however they can be varied to suit different machine-specific protocols.

Cycles	Temp.	Time	Notes
1	95°C	*2 - 5 min	Polymerase activation
40	95°C	10 sec	Denaturation
	60°C	**20 - 50 sec	Annealing/extension (acquire at end of step)

**2 min for cDNA, up to 5 min for genomic DNA.*
***Up to 50 sec may be necessary for multiplexing with more than two probes.*

11. Troubleshooting guide

Problem	Possible Cause	Recommendation
No amplification trace AND No product on agarose gel	Activation time too short	For cDNA templates, make sure qPCR & Go Probe No-ROX is activated for 2 min at 95°C before cycling. For more complex templates such as genomic DNA, increase activation time up to 5 minutes
	Error in protocol setup	Verify that correct reagent concentrations, volumes, dilutions and storage conditions have been used
	Suboptimal primer design	Use primer/probe design software or validated primers. Test primers on a control template

Problem	Possible Cause	Recommendation
No amplification trace AND No product on agarose gel	Incorrect concentration of primers/probe	Use primer concentration between 300 nM and 1 µM and probe concentration of 100 nM
	Template degraded	Re-isolate your template from the sample material or use freshly prepared template dilution
	Primers/probe degraded	Use newly synthesized primers and probe
	Template contaminated with PCR inhibitors	Further dilute template before PCR or purify template and resuspend it in PCR-grade water
	Template concentration too low	Increase concentration used
	Cycling conditions not optimal	Increase extension/annealing times, increase cycle number
No amplification trace AND PCR product present on agarose gel	Error in instrument setup	Check that the acquisition settings are correct during cycling
Non-specific amplification product AND Primer-dimers	Suboptimal primer/probe design	Use primer/probe design software or validated assays. Test primer/probe on a control template
	Primer/probe concentration too high	Test dilution series of primer concentrations until primer dimer/non-specific amplification products disappear
	Primer/probe concentration too low	Use primer concentration between 300 nM and 1 µM and probe concentration of 100 nM
	Primer annealing/extension temperature(s) too low	Due to the high ionic strength of qPCR & Go Probe No-ROX Kit it is not recommended to use annealing/extension temperatures below 60 °C. Annealing/extension temperature can be increased in steps of 2 °C in the event of non-specific products
	Template concentration too low	Increase template concentration
	Template concentration too high	Reduce template concentration until non-specific products disappear
	Extension time too long	Reduce extension time to determine whether non-specific products are reduced

Problem	Possible Cause	Recommendation
Variability between replicates	Error in reaction set-up	Prepare large volume mastermix, vortex thoroughly and aliquot into reaction plate
	Air bubbles in reaction mix	Centrifuge reaction samples/plate prior to running on a real-time instrument
Late amplification trace	Activation time too short	Ensure the reaction is activated for between 2 min and 5 min at 95°C before cycling
	Extension time too short	Increasing the extension time may be necessary for amplification products over 200 bp; double extension time to determine whether the cycle threshold (C_t) is affected
	Annealing temperature too high	Decrease annealing temperature in steps of 2°C
	Template concentration too low	Increase concentration if possible
	Template is degraded	Re-isolate template from sample material or use freshly prepared template dilution
	Suboptimal design of primers	Redesign primers using appropriate software or use validated primers
	Primer/probe concentration too low	Use primer concentration between 300 nM and 1 µM and probe concentration of 100 nM
PCR efficiency below 90%	Extension time is too short	Increase extension time
	Primer/probe concentration too low	Use primer concentration between 300 nM and 1 µM and probe concentration of 100 nM
	Suboptimal design of primers/ probe	Use primer/probe design software or validated assays. Test primer/probe on a control template

12. Associated products

Product	Description	Pack Size	Cat. No
qPCR & Go SYBR® No-ROX Kit	SYBR® Green Real-Time PCR Master Mixes	500 Reactions	11EBI03050
		2000 Reactions	11EBI03200
cDNA Synthesis & Go Kit	First Strand cDNA Synthesis Kit	50 Reactions	11EBI00005

NOTE:

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