

qPCR MasterMix Plus Low ROX Technical Data Sheet

Reference: RT-QP2X-03+WOULR

Products and procedures described in this protocol are intended for research purposes only.

Storage conditions

For long term storage the qPCR MasterMix Plus Low ROX should be stored at -15 °C to -25 °C in a constant temperature freezer. When stored under these conditions the reagents are stable for 2 years.

For short term storage the qPCR MasterMix Plus Low ROX can be stored at 4 °C to 6 °C for 1 month.

Kit contents

The qPCR MasterMix Plus Low ROX contains enough reagents for up to 300 - 50 µl reactions using the hotstart enzyme, HotGoldStar.

Reagent	Volume	Description
2x reaction buffer (red cap)	7.5 ml	One bottle of reaction buffer, dNTPs (including dUTP), HotGoldStar DNA polymerase, MgCl ₂ (5mM final concentration), stabilizers, passive reference
50 mM MgCl ₂ (plain cap)	1.5 ml	One tube of 50 mM MgCl ₂

Procedure

1- Thaw all required reagents completely and put them on ice. Mix all reagents well by inversion and spin them down prior to pipeting.

2- Prepare the reaction mix

Component	Volume (µl)	Final concentration
2x reaction buffer	25	1x
Forward primer	5	(starting with 300 nM)*
Reverse primer	5	(starting with 300 nM)*
Probe	5	(starting with 100 nM)*
Template	5	-
Water	5	(volume is 50 µl minus all other components)
Total Mix	50 µl	

*Note 1: the primer and probe concentrations are recommended as starting concentrations. These concentrations will be correct for many assays, but additional optimization of the primer concentrations and primer-probe ratios may be required to obtain the best results with your primer-probe set.

Note 2: Uracil-N-Glycosylase (reference RT-0610-03) can be added to a final concentration of 0.01 u/µl (0.5 µl of 1 u/µl UNG per 50 µl reaction). If UNG is required, please add the following step in point 6 before the HotGoldStar activation step: 2 min at 50 °C.

3- To correct for dispensing losses prepare an excess of reaction mix (for example 100 reactions reaction mix for 96 reactions). Add all components together, except for the template. Mix thoroughly by inversion. Spin down.

4- Pipette 5 µl of the template DNA for your samples, 5 µl of the control DNA for your positive control and 5 µl of water or buffer for your negative control in to your PCR tubes / 96-well plate / 384-well plate.

5- Add 45 µl of the reaction mix to the reaction vial, close the vial and mix gently on a stirrer or spin down. Ensure that no bubbles are present in the reaction vial. Reaction set up can be done at room temperature.

6- Program the Real-Time thermocycler using the following recommended parameters:

HotGoldStar activation	10 min. 95 °C
40 Cycles	15 sec. 95 °C 1 min. 60 °C
Hold	50 °C forever

Technical information

Primer and probe design guidelines

Probes:

- avoid runs of identical nucleotides, especially of 4 or more Gs
- when using Primer Express® software, the T_m should be 65 °C to 67 °C
- avoid 5' end G (quenches the fluorophore)
- for genotyping, position of the polymorphism should be in the center of the probe, and the probe length should be adjusted such that both probes have the same T_m

Primers:

- GC content should be between 30 % and 80 % (ideally 40-60 %)
- avoid runs of identical nucleotides, especially of 3 or more Gs or Cs at the 3' end
- using the Primer Express® software the T_m should be 58 °C to 60 °C
- the primer should be placed as close as possible to the probe

Custom assay design

Commonly used concentrations are 300 nM for primers and 100 nM for probes. Optimal results may require titration of primers and probes or adjustment of the primer / probe ratio. The purpose of such a process is to determine the minimum amount of primers and probe required to obtain the most sensitive results with your assay.

Primer titration matrix

Titrate according to the Table 1, perform qPCR and select the concentration, which gives the lowest Ct value.

By doing this type of titration it is also possible to compensate for differences up to 2 °C in melt temperature of the primers.

Table 1: Primer titration matrix

Reverse	Forward		
	50 nM	300 nM	900 nM
50 nM	50 / 50	300 / 50	900 / 50
300 nM	50 / 300	300 / 300	900 / 300

Primer-probe ratio matrix

Select optimal primer concentration as described in Table 1 and test with all probe concentrations described in Table 2. Select the concentration, which gives the lowest Ct value

Table 2: Primer-probe ratio matrix

	Probe		
	50 nM	100 nM	250 nM
Opt. primers	50 / opt	100 / opt	250 / opt

MgCl₂ adjustment matrix

Standard MgCl₂ concentration is 5 mM but optimal MgCl₂ concentration can vary between assay, if necessary use the 50 mM MgCl₂ tube. Always prefer optimizing the primer and probe concentrations before the MgCl₂ concentration. Adjust the amount of water if MgCl₂ is added to the reaction.

Final MgCl ₂ concentration (mM)	MgCl ₂ to add (µl/50 µl)	2x reaction buffer (µl)
5	0	25
5.5	0.5	25
6	1	25
6.5	1.5	25
7	2	25
7.5	2.5	25
8	3	25

3-step protocol instead of 2-step protocol

Increasing extension time or performing a 3-step protocol can increase the ΔR_n and / or decrease the Ct of an assay, particularly when the PCR product is longer than 100 bp.

The protocol will be as follows:

HotGoldStar activation		10 min. 95 °C
40 Cycles	denaturation	15 sec. 95 °C
	annealing	20 sec. 60 °C
	extension	40 sec. 72 °C

Increase extension time with 10-second steps, if required.

Further information available through Eurogentec web site, www.eurogentec.com.

- Troubleshooting Guide for qPCR and RTqPCR (under the "Technical Resources / Troubleshooting Guide" section).
- Primers and probe design (please refer to our Troubleshooting Guide).
- "Your One-stop-shop Real-Time qPCR supplier" handbook (under the "Technical Resources / Documentation" section).
- MSDSs, (under the "Technical Resources / MSDS" section)
- Certificates of Analysis (please contact us).

For any further information required please contact our Customer Help Desk:

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