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www.eurogentec.com**qPCR MasterMix Plus w/o UNG 5 ml, 10 pack****10-QP2X-02WOU+**

Eurogentec products are sold for research or laboratory use only and are not to be administrated to humans or used for medical diagnostics.

Storage conditions

For long term storage the qPCR MasterMix Plus 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 can be stored at 4 °C to 6 °C for 4 months.

Kit contents

The qPCR MasterMix Plus 5 ml, 10 pack contains enough reagents for up to 4000 - 25 µl reactions using the hotstart enzyme, HotGoldStar.

Reagent	Volume	Description
2x reaction buffer (green cap)	10 x 5 ml	Ten bottles (5 ml) of reaction buffer, dNTPs (including dUTP), HotGoldStar DNA polymerase, MgCl ₂ (5mM final concentration), stabilizers and passive reference
50 mM MgCl₂ (plain cap)	10 x 1.5 ml	Ten tubes (1.5 ml) 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 pipetting.
- 2- **Prepare the reaction mix** (see table). To correct for dispensing losses prepare an excess of reaction mix (for example, a 100 reactions mix for 96 reactions).
- 3- Add all components together, except for the template. Mix thoroughly by inversion. Spin down.

Component ³	Volume (µl)	Final Concentration
2x reaction buffer	12.5	1x
Forward primer	2.5	(starting with 300 nM) ¹
Reverse primer	2.5	(starting with 300 nM) ¹
Probe	2.5	(starting with 100 nM) ¹
Water	2.5	(volume is 25 µl minus all other components) ²
Total Mix / reaction	22.5 µl²	

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 ratio may be required to obtain the best results with your primer-probe set (see technical information below).

Note 2: 22.5 µl of reaction mix is added to 2.5 µl of template/control DNA prior to cycling, giving a final reaction volume of 25 µl. See steps 4 and 5 below. These volumes can be adjusted depending on the template and reaction volumes.

Note 3: 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.

4- Pipette 2.5 µl of the template cDNA/DNA for your samples, 2.5 µl of the control DNA for your positive control or 2.5 µl of water/buffer for your negative control into your qPCR tubes/plate.

5- Add 22.5 µl of the reaction mix per well / vial, close the plate/vial and mix gently on a stirrer and spin down. Ensure that no bubbles are present in the reaction wells / vials. Reaction set up can be done at room temperature.

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

	Time	T °C
HotGoldStar activation	10 min.	95 °C
40 Cycles		
Denaturation	15 sec.	95 °C
Annealing / extension⁴	45 sec.	60 °C⁴
Hold	∞	50 °C

Note 4: The annealing temperature will vary depending on the melting temperature (T_m) of the primers. Some assays may require longer extension times for efficient amplification. Increase extension time by increments of 5-second, if required.

Technical information**Primer and probe design guidelines**

Probes:

- avoid runs of identical nucleotides, especially of 4 or more Gs
- the probe T_m should 7 to 10 °C above primers T_m.
- 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
- the T_m should be between 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.

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Tel.: +1 858 793 2661 • Fax: +1 858 793 2666 • info.usa@eurogentec.com
usa.eurogentec.com*Primer titration matrix*

Titrate according to the Table 1, perform qPCR and select the concentration which gives the lowest Cq 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
900 nM	50 / 900	300 / 900	900 / 900

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 Cq 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 assays. If necessary use the provided 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/25 µl)	2x reaction buffer (µl)
5	0	12.5
5.5	0.25	12.5
6	0.5	12.5
6.5	0.75	12.5
7	1	12.5

3-step protocol instead of 2-step protocol

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

The protocol will be as follows:

	Time	T°C
HotGoldStar activation / UNG inactivation	10 min.	95 °C
40 Cycles		
Denaturation	15 sec.	95 °C
Annealing ⁴	20 sec.	60 °C ⁴
Extension	40 sec.	72 °C

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

Note 4: The annealing temperature will vary depending on the melting temperature (T_m) of the primers.

For further information please contact our Customer Help Desk:

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