

FAST qPCR MasterMix Plus Low ROX Technical Data Sheet

Reference: RT-QP2X-03+WOULRF

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

Storage conditions

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

The FAST qPCR MasterMix Plus Low ROX reaction buffer should be protected from light whenever possible to avoid degradation of the ROX passive reference.

Kit contents

The FAST qPCR MasterMix Plus Low ROX contains enough reagents for up to 600 - 25 µl reactions using the hotstart enzyme, Meteor *Taq*.

Reagent	Volume	Description
2x reaction buffer (red cap)	7,5 ml	One bottle of reaction buffer, dNTPs (including dUTP), Meteor <i>Taq</i> DNA polymerase, MgCl ₂ (4mM final concentration), ROX passive reference and stabilizers.
50 mM MgCl ₂ (clear 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

To correct for dispensing losses prepare an excess of reaction mix (for example, a 100 reactions mix for 96 reactions).

Component	Volume (µl)	Final Concentration
2x reaction buffer	12.5	1x
Forward primer	2.5	(starting with 300 nM)*
Reverse primer	2.5	(starting with 300 nM)*
Probe	2.5	(starting with 100 nM)*
Water	2.5**	(volume is 25 µl minus all other components)**
Total Mix / reaction	22.5 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 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 if required (0.25 µl of 1 U/µl UNG per 25 µl reaction). If the UNG is required please add the following step at point 6 before the Meteor *Taq* activation step: 2 min. at 50 °C.

3- Add all components together, except for the template. Mix thoroughly by inversion. Spin down.

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 in to your PCR tubes / 96-well plate / 384-well plate.

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

6- Program your Real-Time Thermocycler w/ “fast block” using the following recommended FAST parameters:

UNG Step (if added to the mix)		2 min. 50 °C
Meteor <i>Taq</i> activation / UNG inactivation		5 min. 95 °C
40 Cycles	denaturation	3 sec. 95 °C
	annealing	30 sec. 60 °C *
	extension	10 sec. 72 °C **

* The annealing temperature will vary depending on the melting temperature (T_m) of the primers. Most FAST thermocyclers can accommodate shorter annealing steps for faster qPCR results. On an ABI 7500 FAST, annealing should be 26 sec. min. for data acquisition.

** Please note that short amplicons (<150bp) are recommended to support FAST cycling conditions. Some assays may require longer extension times for efficient amplification. Increase extension time by increments of 5-second, if required.

Standard 2-steps FAST protocols may also be used, provided that the 5 min. 95 °C Meteor *Taq* activation step is maintained. However, performing a 3-steps protocol can increase the ΔRn and / or decrease the Ct of an assay, particularly when the PCR product is longer than 100 bp.

Technical information

Primer and probe design guidelines

Probes:

- avoid runs of identical nucleotides, especially of 4 or more Gs
- the probe T_m should be 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.

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
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 Ct value.

Table 2: Primer-probe ratio matrix

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

MgCl₂ adjustment matrix

Standard MgCl₂ concentration is 4 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)
4	0	12.5
4.5	0.25	12.5
5	0.5	12.5
5.5	0.75	12.5
6	1	12.5

Regular non-FAST protocols

The FAST qPCR MasterMix Plus Low Rox will produce consistent and sensitive results also under regular cycling conditions (standard ramping rates).

Standard protocol will be as follow.

UNG step (if added to the mix)		2 min. 50 °C
Meteor Taq activation / UNG inactivation		5 min. 95 °C
40 Cycles	denaturation	15 sec. 95 °C
	annealing	45-60 sec. 60 °C

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

- Manual for qPCR MasterMix Plus, reference RT-0000-04 (under the "Technical Resources / Manual" section).

- Troubleshooting Guide for qPCR and qRT-PCR (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:

For Europe:

E-mail: info@eurogentec.com

Tel : +32 4 372 76 65

For USA:

E-mail: info.usa@eurogentec.com

Tel: +1 858 793 26 61

FOR RESEARCH USE ONLY NOTICE TO PURCHASER: LIMITED LICENSE

Licensed dsDNA-Binding Dye Research Kits
Use of this product is covered by one or more of the following US patents and corresponding patent claims outside the US:
5,079,352; 5,789,224; 5,618,711; 6,127,155; 5,677,152; 5,773,258; 5,407,800; 5,322,770; 5,310,652; 5,994,056; 6,171,785; and claims outside the US corresponding to US Patent No. 4,859,819. The purchase of this product includes a limited, non-transferable immunity from suit under the foregoing patent claims for using only this amount of product for the purchaser's own internal research. No right under any other patent claim (such as apparatus or system claims in US Patent No. 6,814,934) and no right to perform commercial services of any kind, including without limitation reporting the results of purchaser's activities for a fee or other commercial consideration, is conveyed expressly, by implication, or by estoppel. This product is for research use only. Diagnostic uses under Roche patents require a separate license from Roche. Further information on purchasing licenses may be obtained by contacting the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA.

Cyclic-substituted unsymmetrical cyanine dyes are covered by U.S. Patents 5,436,134 and 5,658,751 and licensed to Eurogentec S.A by Molecular Probes, Inc in the direct research field.

Use of UDG employs U.S. Patents 5,035,936; 5,945,313; 5,683,896 and their foreign counterparts licensed to Eurogentec, S.A from Invitrogen Corporation.

Primer Express® is a registered trademark of Applied Biosystems Corporation.