

One step RT qPCR MasterMix Plus Technical Data Sheet

Reference: RT-QPRT-032X

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

Storage conditions

For long term storage the One step RT qPCR MasterMix Plus should be stored at -65 °C to -75 °C in a constant temperature freezer. When stored under these conditions the reagents are stable for 1 year.

For mid term storage the One step RT qPCR MasterMix Plus can be stored at -15 °C to -25 °C for 6 months.

For short term storage all components of the One step RT qPCR MasterMix Plus, except the tube of Euroscript / RNase Inhibitor, can be stored at 4 °C for one month. The Euroscript / RNase Inhibitor has to be stored at -15 °C to -25 °C.

Kit contents

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

Reagent	Volume	Description
EuroScript RT (white cap)	75 µl	One tube of Moloney Murine leukemia virus RT, 3750 U at 50 U/µl and RNase Inhibitor, 1500 U at 20 U/µl
2x reaction buffer (red cap)	7.5 ml	5 tubes (1,5 ml each) of reaction buffer, dNTPs (including dUTP), HotGoldStar DNA polymerase, MgCl ₂ (5mM final concentration), stabilizers and 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, except the EuroScript reverse transcriptase and RNase Inhibitor, which should be kept in the freezer until required for use. 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*
EuroScript RT & RNase Inhibitor	0.25	0.25 U/ml 0.1 U/ml
Template	variable	10 pg - 100 ng total RNA
RNase free water	Variable	NA (to make up reaction)
Total Mix	50 µl	

*Note that the primer and probe concentrations are recommended as starting concentrations; always start at the lower end. These concentrations will be correct for many assays, but additional optimization may be required to obtain the best results with your primer-probe set.

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- Add the reaction mix to the reaction vial. Reaction set up should be done on ice.

5- Add the template to individual reactions, gently mix on a magnetic stirrer and centrifuge to avoid bubbles. negative control containing no RNA template should always be included. Optionally, a no RT-control should be set up in tubes / wells, which does not contain the EuroScript RT / RNase Inhibitor.

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

Reverse transcription step	30 min. 48 °C
HotGoldStar activation	10 min. 95 °C
EuroScript inactivation	
40 Cycles	15 sec. 95 °C 1 min. 60 °C

Technical information

The use of Uracil-N-glycosylase (UNG) in a one step RT qPCR kit containing dUTPs is **NOT** recommended, as the cDNA will be degraded upon reverse transcription. However, the one step RT qPCR MasterMix Plus does contain dUTPs, to enable the use of UNG for carry over prevention for future reactions.

Primer design guidelines

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

Custom assay design

Commonly used concentrations are 300 nM for primers. Optimal results may require titration of primers. The purpose of such a process is to determine the minimum amount of primers 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 5 mM but optimal MgCl₂ concentration can vary between assay, if necessary use the 50 mM MgCl₂ tube. Always prefer optimizing the primer 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

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:

Reverse transcriptase step		30 min. 48 °C
HotGoldStar activation / RT inactivation		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.

- Manual for One step RT qPCR MasterMix Plus, reference RT-0000-03 (under the "Technical Resources / Manual" section).
- 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:

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 66

FOR RESEARCH USE ONLY

NOTICE TO PURCHASER: LIMITED LICENSE

A license under U.S. Patents 4,683,202, 4,683,195 and 4,965,188 or their foreign counterparts, owned by Roche Molecular Systems, Inc. and F. Hoffmann-La Roche Ltd (Roche), has an up-front fee component and a running-royalty component. The purchase price of this product includes limited, non-transferable rights under the running-royalty component to use only this amount of the product to practice the Polymerase Chain Reaction ("PCR") and related processes described in said patents solely for the research and development activities of the purchaser when this product is used in conjunction with a thermal cycler whose use is covered by the up-front fee component. Rights to the up-front fee component must be obtained by the end user in order to have a complete license. These rights under the up-front fee component may be purchased from Applied Biosystems or obtained by purchasing an Authorized Thermal Cycler. No right to perform or offer commercial services of any kind using PCR, including without limitation reporting the results of purchaser's activities for a fee or other commercial consideration, is hereby granted by implication or estoppel. Further information on purchasing licenses to practice the PCR Process may be obtained by contacting the Director of Licensing at Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404 or the Licensing Department at Roche Molecular Systems, Inc., 1145 Atlantic Avenue, Alameda, California 94501.

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.

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