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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, 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	0.25	0.25 U/ml
& RNase Inhibitor		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 bubles. 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 **EuroScript inactivation**	10 min. 95 °C
40 Cycles	15 sec. 95 °C 1 min. 60 °C

Technical information

The use of Uracil-N-glicosylase (UNG) in a one step RT qPCR kit is **NOT** recommended, as the cDNA will be degraded upon reverse transcription.

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 or Cs at the 3'end
- using the Primer Express® software the Tm should be 58 °C to 60 °C

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

	Forward		
Reverse	**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

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

$MgCl_2$ adjustment matrix
Standard $MgCl_2$ concentration is 5 mM but optimal $MgCl_2$ concentration can vary between assay, if necessary use the 50 mM $MgCl_2$ tube. Always prefer optimizing the primer concentrations before the $MgCl_2$ concentration.
Adjust the amount of water if $MgCl_2$ 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 ÄRn 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).

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