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MESA GREEN qPCR MasterMix Plus for SYBR® Assay I dTTP Technical Data Sheet

Reference: RT-SY2X-03+WOUN

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

Storage conditions

For long term storage the MESA GREEN qPCR MasterMix Plus for SYBR® Assay I dTTP should be stored at -15 °C to -25 °C in a constant temperature freezer. When stored under these conditions the reagents are stable for 1 year.

For short term storage the MESA GREEN qPCR MasterMix Plus for SYBR® Assay I dTTP can be stored at 4 °C to 6 °C for 1 month.

The MESA GREEN qPCR MasterMix Plus for SYBR® Assay I dTTP reaction buffer should be protected from light whenever possible.

Kit contents

The MESA GREEN qPCR MasterMix Plus for SYBR® Assay dTTP contains enough reagents for up to 300 - 50 µl reactions using the hotstart enzyme, Meteor*Taq*.

Reagent	Volume	Description
2x reaction buffer (yellow cap) (amber bottle)*	7.5 ml	One bottle of reaction buffer, dNTPs, Meteor*Taq* DNA polymerase, $MgCl_2$ (4mM final concentration) SYBR® Green I, stabilizers and passive reference
50 mM $MgCl_2$ (clear cap)	1,5 ml	One bottle of 50 mM $MgCl_2$

*The SYBR® Green I included in the 2x reaction buffer is light sensitive and should be kept away from light as much as possible.

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	(initially 100 up to 300 nM)*
Reverse primer	5	(initially 100 up to 300 nM)*
Template	5	-
Water	10	(volume is 50 µl minus all other components)
Total Mix	**50 µl**	

*Note 1: the primer 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 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- 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.

6a- If the Real Thermocycler used has a **"normal" block**, program it using the following recommended parameters:

Meor*Taq* activation	5 min. 95 °C
40 Cycles	15 sec. 95 °C 1 min. 60 °C
Hold at 50 °C forever or perform a meltcurve	

6b- If the Real Thermocycler used has a **"fast" block**, then it is necessary to program it using the following recommended parameters:

Meor*Taq* activation	5 min. 95 °C
40/45 Cycles	3 sec. 95 °C 45-60 sec. 60 °C
Hold at 50 °C forever or perform a meltcurve	

Technical information

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 100 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**	**100 nM**	**300 nM**
50 nM	50 / 50	100 / 50	300 / 50
100 nM	50 / 100	100 / 100	300 / 100
300 nM	50 / 300	100 / 300	300 / 300

$MgCl_2$ adjustment matrix

Standard $MgCl_2$ concentration is 4 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)**
4	0	25
4.5	0.5	25
5	1	25
5.5	1.5	25
6	2	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:

Meteor*Taq* activation		5 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).

- MSDS, (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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