

MESA Blue qPCR MasterMix Plus for SYBR® Assay w/ fluorescein

Technical Data Sheet

Reference: RT-SY2X-03+WOUFLB

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

Storage conditions

For long term storage the MESA Blue qPCR MasterMix Plus for SYBR® Assay w/ fluorescein 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 Blue qPCR MasterMix Plus for SYBR® Assay w/ fluorescein can be stored at 4 °C to 6 °C for 1 month.

The MESA Blue qPCR MasterMix Plus for SYBR® Assay w/ fluorescein reaction buffer should be protected from light whenever possible.

Kit contents

The MESA Blue qPCR MasterMix Plus for SYBR® Assay w/ fluorescein contains enough reagents for up to 300 - 50 µl reactions using the hotstart enzyme, MeteorTaq.

Reagent	Volume	Description
2x reaction buffer (blue cap) (amber bottle)*	7,5 ml	One bottle of reaction buffer, dNTPs (including dUTP) MeteorTaq DNA polymerase, MgCl ₂ (4mM final concentration), SYBR® Green I, Blue dye, fluorescein and stabilizers.
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

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

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)*
Water	10**	(volume is 50 µl minus all other components)**
Total Mix / reaction	45 µl**	

*Note 1: the primer concentrations are recommended as starting concentrations. These concentrations will be correct for many assays, but additional optimization of the primer concentrations may be required to obtain the best results with your primer set (see technical information below).

**Note 2: 45µl of reaction mix is added to 5 µl of template/control DNA prior to cycling, giving a final reaction volume of 50 µl. See steps 4 and 5 below. These volumes can be adjusted depending on the total reaction volume.

***Note 3: Uracil-N-Glycosylase (reference RT-0610-03) can be added to a final concentration of 0.01 U/µl if required (0.5 µl of 1 U/µl UNG per 50 µl reaction). If the UNG is required please add the following step at point 6 before the MeteorTaq activation step: 2 min. at 50 °C.

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

4- Pipette 5 µl of the template cDNA/DNA for your samples, 5 µl of the control DNA for your positive control or 5 µl of water/buffer for your negative control in to your PCR tubes / 96-well plate / 384-well plate.

5- Add 45 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 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:

MeteorTaq 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 possible to program it using the following recommended parameters:

MeteorTaq 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
- The T_m should be between 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

Reverse	Forward		
	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

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 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)
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 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:

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 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).
- 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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