EURx® MOLECULAR BIOLOGY PRODUCTS

MP qPCR Master Mix (2x)

Kit Components

Component	Cat. No. E0424-01 100 reactions of 25 µl	Cat. No. E0424-02 200 reactions of 25 µl	Cat. No. E0424-03 1000 reactions of 25 µl
MP qPCR Master Mix (2x)	1 x 1.25 ml	2 x 1.25 ml	10 x 1.25 ml
Thermolabile UNG (uracil-N-glycosylase) 1 U/µl	30 µl	55 µl	270 µl
Water, nuclease free	1 x 1.25 ml	2 x 1.25 ml	10 x 1.25 ml

MP qPCR Master Mix (2x), plus ROX Solution

Kit Components

Component	Cat. No. E0425-01 100 reactions of 25 µl	Cat. No. E0425-02 200 reactions of 25 µl	Cat. No. E0425-03 1000 reactions of 25 µl
MP qPCR Master Mix (2x)	1 x 1.25 ml	2 x 1.25 ml	10 x 1.25 ml
ROX Solution, 25 µM	55 µl	110 µl	530 µl
Thermolabile UNG (uracil-N-glycosylase) 1 U/µl	30 µl	55 µl	270 µl
Water, nuclease free	1 x 1.25 ml	2 x 1.25 ml	10 x 1.25 ml

Storage

Store at -20°C in the dark for long-term storage or at 4°C for up to 2 weeks.

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Description

- MP qPCR Master Mix (2x) is a universal solution for quantitative, multiplex real-time PCR and two-step real-time RT-PCR using sequence-specific probes and can be used on most real-time PCR cyclers available.
- Depending on the real-time cycler used, up to 5 targets can be quantified simultaneously in the same well or tube.
- The master mix contains onTaq DNA Polymerase, optimized reaction buffer, dNTPs (dTTP is partially replaced with dUTP).
- onTaq DNA Polymerase is a modified „hot start" enzyme that is blocked at moderate temperatures and allows room temperature reaction setup. The polymerase activity is restored during 15 min initial denaturation step at 95°C.
- Use of the "hot start" enzyme prevents extension of misprimed products and primer-dimers during reaction setup leading to higher specificity and sensitivity of PCR reactions.
- The polymerase enables convenient room temperature reaction setup.
- MP qPCR Master Mix (2x) contains dUTP, which partially replaces dTTP. It allows the optional use of a thermolabile uracil-N glycosylase (UNG) to prevent carryover contamination between reactions. UNG removes uracil from any dU-containing contaminating amplicons, leaving abasic sites and making DNA molecules susceptible to hydrolysis during the initial denaturation step.
- There are two variants of the kit: without ROX and with ROX Solution provided separately. The use of ROX passive reference dye is necessary for all real-time PCR cyclers from Applied Biosystems and optional for cyclers from Stratagene. ROX compensates for variations of fluorescent signal between wells due to slight differences in reaction volume and fluorescence fluctuations. ROX is not involved in PCR reaction and does not interfere with real-time PCR on any instrument. Refer to the table below to determine the recommended amount of ROX (25 µM) required for a specific PCR cycler.

Recommended amounts of ROX for a specific real-time PCR cycler

Instrument	Amount of ROX per 25 µl reaction	Amount of ROX per 1.25 ml of 2x master mix	Final ROX concentration
Applied Biosystems: 7300, 7900HT, StepOne, StepOnePlus, ABI PRISM 7000 and 7700	0.5 µl	50 µl	500 nM
Applied Biosystems: 7500, ViiA 7, Stratagene: Mx3000P, Mx3005P, Mx4000	0.5 µl 10 x diluted (in water)	50 µl 10 x diluted (in water)	50 nM
PCR machines from other manufacturers: Bio-Rad, Roche, Corbett, Eppendorf, Cepheid, etc.	Not required	Not required	-

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Protocol

Preparation of PCR Reaction:

Component	Volume/reaction	Final concentration
MP qPCR Master Mix (2x)	12.5 µl	1 x 4.5 mM $MgCl_2$
20 x primer-probe mix 1	1.25 µl	0.2-0.4µM forward primer 1 0.2-0.4µM reverse primer 1 0.2 µM probe 1
20 x primer-probe mix 2	1.25 µl	0.2-0.4 µM forward primer 2 0.2-0.4µM reverse primer 2 0.2 µM probe 2
Optional: 20 x primer-probe mix 3 and 4	1.25 µl	0.2 µM forward primer 3, 4 0.2 µM reverse primer 3, 4 0.2 µM probe 3, 4
Template DNA	Variable	≤500 ng
Optional: ROX Solution, 25 µM	0.5 µl or 0.5 µl 10 x diluted	500 nM 50 nM
Optional: Thermolabile UNG (uracil-N-glycosylase) 1 U/µl	0.25 µl	0.25 U/reaction
Water, nuclease free	To 25 µl	-
Total volume	25 µl	-

Notes:

1. A reaction volume of 25 µl should be used with most real-time cyclers. Other reaction volumes may be used if recommended for a specific instrument.
2. Optimal amplicon length in real-time PCR using probes is 70-200 bp.
3. Thaw, gently vortex and briefly centrifuge all solutions.
4. Set up PCR reactions at room temperature. Use of MP qPCR Master Mix (2x) allows room temperature reaction setup.
5. Prepare a reaction master mix by adding all the reaction components except template DNA.
6. Mix the reaction mix thoroughly and dispense appropriate volumes into PCR tubes or plates.
7. Add template DNA/cDNA (≤500 ng/reaction) to the individual PCR tubes or wells containing the reaction mix. For two-step RT-PCR, the volume of cDNA added should not exceed 10% of the final PCR volume.
8. Centrifuge briefly to settle down the reaction components and remove bubbles. Bubbles interfere with fluorescent detection.
9. Place the samples in the cycler and start the program.
10. $MgCl_2$ concentration provided with the 1 x MP qPCR Master Mix is 4.5 mM. In most cases this concentration will produce optimal results. However, if a higher $MgCl_2$ concentration is required, prepare a 25 mM $MgCl_2$ stock solution and add to a reaction.
11. For duplex PCR a final primer concentration of 0.4 µM is usually optimal, while for triplex and 4-plex PCR the recommended starting concentration is 0.2 µM for all primers. Raising primer concentration may increase PCR efficiency, but negatively affect PCR specificity. Optimal primer concentration depends on the individual reaction and the real-time PCR cycler used. For 4-plex PCR and large differences in Ct values between the individual targets (e.g. cDNA targets) better results (stronger fluorescence signals for targets with high Ct) can be obtained by varying the concentration of the primers: 0.4 µM for targets with high Ct and 0.1 µM for targets with low Ct.
12. A final probe concentration of 0.2 µM gives satisfactory results in most cases. Depending on the synthesis quality and the purification method used, the optimal concentration may be in range of 0.1 µM to 0.4 µM.
13. Optimal melting temperature (T_m) of primers should be near 60°C. The T_m of dual-labeled probes should be 8-10°C higher than the T_m of the primers.
14. Avoid G at the 5'-end of the dual-labeled probe, which causes quenching of fluorescence signal.
15. Perform data analysis.

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Thermal Cycling Conditions:

Step	Temperature	Time	Number of Cycles
Optional: Thermolabile UNG pre-treatment	37°C	2 min	1
Initial Denaturation	95°C	15 min	1
Denaturation	95°C	15 s	40-50
Annealing/Extension	60°C	60 s	
Cooling	4°C	Indefinite	1

Notes:

1. MP qPCR Master Mix (2x) has been developed for use in a two-step cycling protocol. This protocol works well for most primers (even for primers with a T_m well below 60 °C).
2. The incubation step of 37°C for 2 minutes must be added if a thermolabile uracil-N-glycosylase is used to prevent carryover contamination. UNG degrades any dUMP-containing PCR products.
3. During the initial denaturation step onTaq DNA Polymerase is activated. onTaq DNA polymerase requires 15 min incubation at 95°C to restore activity.
4. It is recommended to check the PCR product specificity by gel electrophoresis when designing a new assay.

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