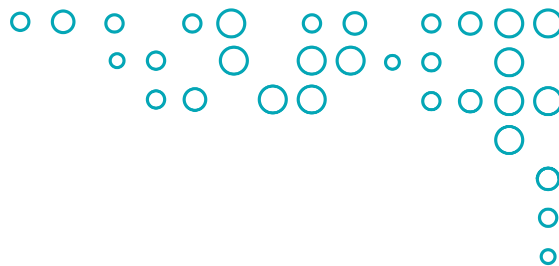
EURx® MOLECULAR BIOLOGY PRODUCTS



Multiplex PCR Master Mix (2x)

Cat. No.	size
E2820-01	50 reactions of 50 µl
E2820-02	100 reactions of 50 µl

Storage Conditions: Store at -20°C for long-term storage or at 4°C for up to 1 month.

Description:

- Multiplex PCR Master Mix (2x) is developed for efficient multiplex PCR for various advanced applications. The use of the kit eliminates the need for time-consuming optimization, making the development of multiplex PCR assays easier and faster.
- Use of Multiplex PCR Master Mix (2x) allows to save time and reduce contamination risk due to fewer pipetting steps during PCR setup.
- The master mix contains onTaq DNA Polymerase, $MgCl_2$, specially formulated reaction buffer, dNTPs and additives.
- onTaq DNA Polymerase is a modified "hot start" enzyme which is blocked at moderate temperatures and allows room temperature reaction setup.
- The polymerase activity is restored during 10 min initial denaturation step.
- 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.
- Multiplex PCR Master Mix (2x) enables convenient room temperature reaction setup.
- The master mix can be used with plain primers or fluorescently labeled primers.

10 x Color Load:

10 x Color Load contains two gel tracking dyes and a gel loading reagent. It enables direct loading of PCR products onto an agarose gel.

Multiplex PCR Master Mix (2x) contains:

Component	50 reactions	100 reactions
Multiplex PCR	1 x 1.3 ml	2 x 1.3 ml
10 x Color Load	0.3 ml	0.6 ml
Water, nuclease free	1 x 1.3 ml	2 x 1.3 ml

This product is developed, designed and sold exclusively for research purposes and in vitro use only.
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Preparation of multiplex PCR Reaction:

Component	Volume/reaction	Final concentration
Multiplex PCR Master Mix (2x)	25 µl	1x 2.5 mM $MgCl_2$
10 x primer mix, 2 µM each primer	5 µl	0.2 µM
Optional: 10 x Color Load	5 µl	1x
Template DNA	Variable	≤0.3 µg/50 µl 1-10 ng (for fluorescently labeled primers)
Sterile double-distilled water	To 50 µl	-
Total volume	50 µl	-

Notes:

1. Thaw, gently vortex and briefly centrifuge all solutions before use to avoid localized differences in salt concentration.
2. Set up PCR reactions at room temperature. Multiplex PCR Master Mix (2x) allows room temperature reaction setup.
3. Prepare a reaction master mix by adding all the reaction components except template DNA.
4. Mix the reaction mix thoroughly and dispense appropriate volumes into PCR tubes or plates.
5. Add template DNA/cDNA (≤300 ng/reaction) to the individual PCR tubes containing the reaction mix. In case of using fluorescently labeled primers and PCR products analysis on capillary sequencing instruments add 1-10 ng of DNA.
6. Centrifuge briefly to settle down the reaction components.
7. Place the samples in the cycler and start the program.
8. The concentration of $MgCl_2$ in 1 x Multiplex PCR Master Mix is 2.5 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.
9. A final primer concentration of 0.2 µM is usually optimal, but can be individually optimized in range of 0.2 µM to 0.4 µM.
10. Use of the 10 x Color Load allows PCR reactions to be loaded directly onto an agarose gel without prior addition of a gel loading buffer. The 10 x Color Load contains a gel loading reagent and two gel tracking dyes (a red dye and a yellow dye) that separate during electrophoresis. In a 1% agarose gel, the red dye migrates at the same rate as 600 bp DNA fragment and the yellow dye migrates faster than 20 bp. The dyes do not interfere with most downstream enzymatic applications, however it is recommended to purify PCR products prior enzymatic manipulation.
11. In most cases there is no need to add additives to the PCR reaction. For some difficult targets such as: GC-rich sequences, sequences with complex secondary structures additives such as DMSO can be included to improve amplification. Use DMSO in concentrations of 2-5%. The recommended starting DMSO concentration (if needed) is 3%.

Thermal Cycling Conditions

A. Plain Primers

Step	Temperature	Time	Numbers of Cycles
Initial Denaturation	95°C	10 min	1
Denaturation	94°C	30 s	35-45
Annealing	56-64°C	90 s	
Extension	72°C	30 s for fragments up to 500 bp 90 s for fragments up to 1.5 kb	
Final Extension	68°C	7 min	1
Cooling	4°C	Indefinite	1

B. Fluorescently Labeled Primers, PCR products analysis on capillary sequencing instruments

Step	Temperature	Time	Number of Cycles
Initial Denaturation	95°C	10 min	1
Denaturation	94°C	30 s	28
Annealing	56-64°C	90 s	
Extension	72°C	30 s for fragments up to 500 bp 90 s for fragments up to 1.5 kb	
Final Extension	60°C	30 min	1
Cooling	4°C	Indefinite	1

Notes:

1. 10 min initial denaturation step at 95°C is required to restore the polymerase activity.
2. Annealing temperature should be optimized for each primer mix based on the lowest T_m. If possible perform a gradient PCR to determine the optimal annealing temperature.
3. Fewer PCR cycles are needed to generate sufficient product for analysis using fluorescence detection instruments. The optimal number of PCR cycles is dependent on the sensitivity of the detection system and should be determined individually as recommended by the instrument manufacturer. Do not exceed 30 cycles (28 cycles is usually optimal).
4. 30 min final extension step at 60°C is required to add an extra A to the 3' end of all PCR products.

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