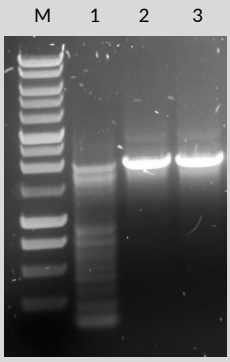
EURx® MOLECULAR BIOLOGY PRODUCTS

onTaq PCR Master Mix (2x)

Cat. No.	size
E2714-01	100 reactions of 50 µl
E2714-02	200 reactions of 50 µl
E2714-03	500 reactions of 50 µl

Storage Conditions: Store at -20°C for long-term storage or at 4°C for up to 2 weeks.



PCR amplification using EURx onTaq PCR Master Mix (2x). A 2 kb amplicon of the human β-globin gene was amplified using onTaq PCR Master Mix (2x).

Lane M: molecular size marker- Perfect Plus 1 kb DNA Ladder (E3131).

Lane 1: PCR amplification reaction using Taq PCR Master Mix (2x). Reaction was incubated 30 min at 25°C before PCR.

Lanes 2, 3: PCR amplification reactions using onTaq PCR Master Mix (2x). Reactions were incubated 30 min at 25°C before PCR.

References:

1. *Chien, A., Edgar, D.B. and Trela, J.M. (1976) J. Bacteriol. 127, 1550.*
2. *Kaledin, A.S., Sliusarenko, A.G. and Gorodetskii, S.I. (1980) Biokhimiya 45, 644.*

Description:

- onTaq PCR Master Mix (2x) is a ready-to-use solution containing onTaq DNA Polymerase, optimized reaction buffer, $MgCl_2$ and dNTPs.
- Use of onTaq PCR Master Mix (2x) allows to save time and reduce contamination risk due to fewer pipetting steps during PCR reaction setup.
- onTaq DNA Polymerase is a modified „hot start" enzymes which provides very tight inhibition of the polymerase activity at moderate temperatures and allows room temperature reactions setup.
- The polymerase activity is restored during the initial denaturation step when amplification reactions are heated at 95°C for 10 minutes.
- "Hot start" PCR may increase specificity, sensitivity and yield of a PCR reaction in comparison to the conventional PCR assembly method.
- onTaq DNA Polymerase replicates DNA at 72°C and exhibits a half-life of 40 min at 95°C (1,2).
- Contains the 5'→3' exonuclease activity.
- Lacks the 3'→5' exonuclease activity.
- Adds extra A at the 3' ends.
- onTaq DNA Polymerase is recommended for use in PCR and primer extension reactions at elevated temperatures to obtain a wide range of DNA products up to 10 kb.

onTaq PCR Master Mix (2x) contains:

- onTaq PCR Master Mix (2x)
- Water, nuclease free
- 10 x Color Load

onTaq PCR Master Mix (2x):

onTaq DNA Polymerase is supplied in 2 x Pol Buffer B containing 3 mM $MgCl_2$ and 0.4 mM of each dNTP.

Quality Control:

All preparations are assayed for contaminating endonuclease, 3'-exonuclease, and nonspecific single- and double-stranded DNase activities. Typical preparations are greater than 95% pure, as judged by SDS polyacrylamide gel electrophoresis.

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Preparation of PCR Reaction:

Component	Volume/reaction	Final concentration
onTaq PCR Master Mix (2x)	25 µl	1.25 U onTaq DNA Polymerase 1 x Reaction Buffer (1.5 mM $MgCl_2$) 0.2 mM of each dNTP
Upstream primer	Variable	0.1-0.5 µM
Downstream primer	Variable	0.1-0.5 µM
Optional: 10 x Color Load	5 µl	1 x
Template DNA	Variable	<0.5 µg/50 µl
Sterile double-distilled water	To 50 µl	-
Total volume	50 µl	-

Notes:

1. Thaw, gently vortex and briefly centrifuge onTaq PCR Master Mix (2x) and primers before use to avoid localized differences in salt concentration.
2. Set up PCR reactions at room temperature. Use of onTaq PCR Master Mix (2x) allows room temperature reaction setup.
3. Primers can be added separately or as a primer mix prepared previously.
4. Vortex the samples and briefly spin down.
5. Reactions can be placed in a room temperature thermal cycler.
6. Standard concentration of $MgCl_2$ in PCR reaction is 1.5 mM (as provided with the 1 x onTaq PCR Master Mix) when using 0.2 mM dNTP (each). In most cases this concentration will produce satisfactory results. However, if a higher $MgCl_2$ concentration is required, prepare a 25 mM $MgCl_2$ stock solution and add to a reaction.
7. 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.
8. 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 2-8%. The recommended starting DMSO concentration (if needed) is 3%.
9. As a general guide for how much template DNA to use, start with a minimum 10^4 copies of the target sequence to obtain a signal in 25-35 cycles (i.e. 1 µg of 1 kb ds DNA equals 9.1 x 10^{11} molecules, 1 µg of *E. coli* genomic DNA equals 2 x 10^8 molecules, 1 µg of human genomic DNA equals 3 x 10^5 molecules).

Thermal Cycling Conditions:

Step	Temperature	Time	Number of Cycles
Initial Denaturation	95°C	10 min	1
Denaturation	94°C	30 s	25-35
Annealing	50-68°C	30 s	
Extension	72°C	1 min/1 kb	
Final Extension	72°C	7 min	1
Cooling	4°C	Indefinite	1

Notes:

1. 10 min initial denaturation step at 95°C is required to activate onTaq DNA Polymerase.
2. Annealing temperature should be optimized for each primer set based on the primer T_m. Optimal annealing temperatures may be above or below the estimated T_m. As a starting point, use an annealing temperature 5°C below T_m.
3. When amplifying long PCR products (over 5 kb), use an elongation temperature of 68°C instead of 72°C.

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