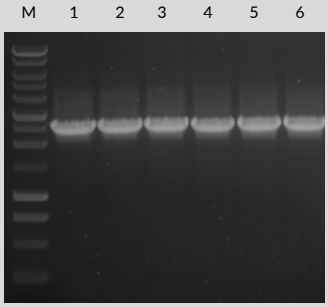
EURx® MOLECULAR BIOLOGY PRODUCTS

Hybrid PCR Master Mix (2x)

Cat. No.	size
E2750-01	100 reations of 50 µl
E2750-02	500 reations of 50 µl

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



PCR amplification using EURx Hybrid PCR Master Mix (2x). A 2.5 kb amplicon of the human CCR5 gene was amplified with Hybrid DNA Polymerase in stand-alone or master mix formats. All reactions were run in 50 µl reaction volume.
Lane M: molecular size marker- Perfect Plus 1 kb DNA Ladder.
Lanes 1,2: PCR amplification reactions using 1 U Hybrid DNA Polymerase, 10 x Hybrid Buffer and dNTPs
Lanes 3,4: PCR amplification reactions using Hybrid PCR Master Mix (2x), after 25 freeze-thaw cycles
Lanes 5,6: PCR amplification reactions using Hybrid PCR Master Mix (2x) and 10 x Color Load, after 25 freeze-thaw cycles

Description:

- Hybrid PCR Master Mix (2x) is a ready-to-use solution containing Hybrid DNA Polymerase, optimized reaction buffer, $MgCl_2$ and dNTPs.
- Use of Hybrid PCR Master Mix (2x) allows to save time and reduce contamination risk due to fewer pipetting steps during PCR set-up.
- Hybrid is a genetically engineered thermophilic DNA polymerase.
- Ultrapure recombinant enzyme.
- The enzyme catalyzes the polymerization of nucleotides into duplex DNA in the 5'→3' direction in the presence of magnesium ions.
- The enzyme exhibits the 3'→5' proofreading activity, resulting in 50-fold higher PCR fidelity than possible with Taq DNA Polymerases.
- The enzyme generates blunt ends.
- Enhanced polymerase processivity allows to use shorter extension times.
- The modification of Hybrid DNA Polymerase enhances target length capability of PfuPlus! DNA Polymerase with regard to genomic targets (up to 12 kb from human genomic DNA).
- **Due to the polymerase modification, the optimal reaction conditions (especially annealing temperatures) differ from standard PCR protocols**.
- Hybrid PCR Master Mix (2x) is recommended for general use in PCR, use in high-fidelity PCR, PCR of GC-rich sequences or problematic secondary structures and cloning of blunt-ended PCR products.

Hybrid PCR Master Mix (2x) contains:

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

Hybrid PCR Master Mix (2x):

Hybrid DNA Polymerase is supplied in 2 x Hybrid Buffer 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.

EURx Ltd. 80-297 Gdańsk Poland ul. Przyrodników 3, NIP 957-07-05-191, KRS 0000202039
www.eurx.com.pl, orders@eurx.com.pl, *tel.* +48 58 524 06 97, *fax* +48 58 341 74 23

Preparation of PCR Reaction:

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

Notes:

1. Thaw, gently vortex and briefly centrifuge Hybrid PCR Master Mix (2x) and primers before use to avoid localized differences in salt concentration.
2. .PCR reactions should be set up on ice.
3. Primers can be added separately or as a primer mix prepared previously.
4. Vortex the samples and briefly spin down.
5. Place reactions in a thermal cycler that has been preheated to denaturation temperature.
6. For Hybrid DNA Polymerase-based PCR, standard concentration of $MgCl_2$ is 1.5 mM (as provided with the 1 x Hybrid PCR Master Mix). In most cases this concentration will produce satisfactory results. For some PCR targets (especially cDNA targets) $MgCl_2$ optimization may be required. If increased Mg^{2+} concentration is needed, 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 PCR reaction. For some difficult targets such as: GC-rich sequences, sequences with complex secondary structures and long targets additives such as DMSO (provided) can be included to improve amplification. The recommended starting DMSO concentration (if needed) is 3%.
9. The amount of DNA template required depends on the type of DNA being amplified. Generally 50-250 ng of genomic DNA, 0.1-10 ng of plasmid DNA, 1-20 ng of phage DNA and 10-100 ng of multicopy chromosomal genes is recommended.

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Thermal Cycling Conditions for Products up to10 kb

Step	2-step protocol		3-step protocol		
	Temp.	Time	Temp.	Time	Number of Cycles
Initial Denaturation	98°C	30 s	98°C	30 s	1
Denaturation	98°C	5-10 s	98°C	5-10 s	25-35
Annealing	-	-	X°C	10-30 s	
Extension					
a. general (genomic targets and episomal targets over 2 kb)	72°C	30 s/1 kb	72°C	30 s/1 kb	
b. episomal targets up to 2 kb	72°C	15-20 s/ 1 kb	72°C	15-20 s/ 1 kb	
Final Extension	72°C	5-7 min	72°C	5-7 min	1
Cooling	4°C	Indefinite	4°C	Indefinite	1

Thermal Cycling Conditions for Products over 10 kb

Step	2-step protocol		3-step protocol		
	Temp.	Time	Temp.	Time	Number of Cycles
Initial Denaturation	92-93°C	2 min	92-93°C	2 min	1
Denaturation	92-93°C	10 s	92-93°C	10 s	25-35
Annealing	-	-	X°C	10-30 s	
Extension	72°C	30 s/1 kb	72°C	30 s/1 kb	
Final Extension	72°C	5-7 min	72°C	5-7 min	1
Cooling	4°C	Indefinite	4°C	Indefinite	1

Notes:

1. A 30-second initial denaturation at 98°C is recommended for most targets up to 10 kb. The initial denaturation time can be extended up to 3 min in case of tempates that require longer denaturation. Denaturation at lower temperatures (92-93°C) allows to achieve higher yield for long targets over 10 kb.
2. Hybrid DNA Polymerase has the ability to stabilize primer-template hybridization. Melting temperatures (T_m) and optimal annealing temperatures usually differ significantly (are higher) from the temperatures calculated/determined for standard PCR polymerases. T_m's should be calculated with the base-stacking method (nearest-neighbor method) using calculator on the website: http://eurx.com.pl/CALC/. Default parameters are: 500 nM primer concentration, 50 mM salt concentration, 1.5 mM Mg^{2+} concentration. As a basic rule use an annealing temperature at a T_m +2°C of the lower T_m primer. In some cases optimal annealing temperatures may differ from the rule given above and should be determined empirically.
3. A 2-step protocol allows to perform combined annealing/ extension step at 72°C and is recommended for primers with T_m values of at least 70°C. The 2-step protocol allows to save time of PCR reaction.
4. Extension time of 30 s/ 1 kb is recommended for most targets. In some cases (episomal targets up to 2 kb) shorter extension time of 15-20 s/ 1 kb may not affect a PCR yield and allow to save amplification time.

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