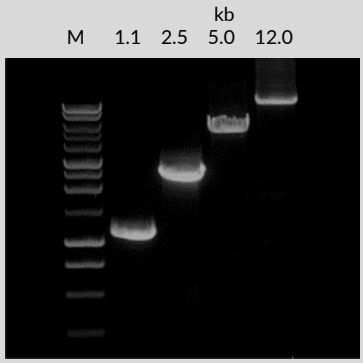
EURx® MOLECULAR BIOLOGY PRODUCTS

Hybrid
DNA Polymerase

Cat. No.	size
E2950-01	100 units
E2950-02	500 units

Unit Definition: One unit is defined as the amount of enzyme required to catalyze the incorporation of 10 nmoles of dNTP into acid-insoluble material in 30 minutes at 74°C. The reaction conditions are: 50 mM Tris-HCl (pH 9.0 at 25°C), 50 mM NaCl, 5 mM $MgCl_2$, 200 µM each of dATP, dCTP, dGTP, dTTP (a mix of unlabeled and [^{3}H]dTTP), 10 µg activated calf thymus DNA and 0.1 mg/ml BSA in a final volume of 50 µl.

Storage Conditions: Store at -20°C.



PCR amplification using EURx Hybrid DNA Polymerase.

Lane M: molecular size marker-Perfect 1 kb DNA Ladder.

Lanes 1.1-12 kb: PCR amplification reactions, using 1 U of EURx Hybrid DNA Polymerase and 100-500 ng of human genomic DNA as a template for 35 cycles in 50 µl reaction volume.

Extremely thermostable DNA polymerase, enabling efficient high fidelity PCR of genomic targets up to 12 kb and episomal targets up to 20 kb.

Description:

- 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.
- Hybrid DNA Polymerase has enhanced target length capability 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 DNA Polymerase 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.

Storage Buffer:

20 mM Tris-HCl (pH 8.0 at 22°C), 100 mM KCl, 0.1 mM EDTA, 1 mM dithiothreitol, 50% glycerol and stabilizers.

10 x Hybrid Buffer:

The buffer contains 15 mM $MgCl_2$.

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
10 x Hybrid Buffer contains 15 mM $MgCl_2$	5 µl	1x
dNTP mix (5 mM each)	2 µl	0.2 mM of each dNTP
Upstream primer	Variable	0.5 µM
Downstream primer	Variable	0.5 µM
Hybrid DNA Polymerase, 2 U/µl	0.5 µl	1 U
Template DNA	Variable	<0.5 µg/50 µl
DMSO, optional	1-5 µl	2-10%
Sterile double-distilled water	Variable	-
Total volume	50 µl	-

Notes:

1. Completely thaw and mix thoroughly all components of PCR reaction before use to avoid localized differences in salt concentration. It is especially important for 10 x Hybrid Buffer, because it may form a precipitate when frozen.
2. Prepare reaction mixes on ice. Mix well.
3. Place reactions in a thermal cycler that has been preheated to denaturation temperature.
4. Hybrid DNA Polymerase should be the last component added to the PCR mixture. In the absence of dNTPs proofreading activity of Hybrid DNA Polymerase may degrade primers.
5. For Hybrid DNA Polymerase-based PCR, standard concentration of $MgCl_2$ is 1.5 mM (as provided with the 1 x Hybrid Buffer). 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 use 25 mM $MgCl_2$ provided to adjust the concentration.
6. 1 U of Hybrid DNA Polymerase is recommended concentration of the enzyme per 50 µl amplification reaction. For most applications, enzyme will be in excess and will produce satisfactory results. For some PCR targets further optimization will be required. Increased amounts of enzyme may generate artifacts like as smearing of bands, etc.
7. High quality dNTPs should be used for optimal performance with Hybrid DNA Polymerase.
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/	72°C	15-20 s/	
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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