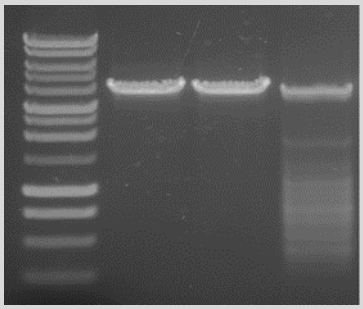
EURx® MOLECULAR BIOLOGY PRODUCTS

onHybrid DNA Polymerase HOT START

Cat. No.	size
E2960-01	100 units
E2960-02	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 onHybrid DNA Polymerase. A 4 kb amplicon of the human β-globin gene was amplified using EURx onHybrid DNA Polymerase, 10 x Hybrid Buffer and 0.2 mM dNTPs in 50 µl reaction volume.
Lane M: molecular size marker – Perfect Plus 1 kb DNA Ladder.
Lane 1, 2: PCR amplification reactions using 1 U onHybrid DNA Polymerase. Reactions were incubated 30 min at 25°C before PCR.
Lane 3: PCR amplification reactions using 1 U Hybrid DNA Polymerase. The reaction was incubated 30 min at 25°C before PCR.

Extremely thermostable hot start DNA polymerase, enabling efficient high fidelity PCR of genomic targets up to 10 kb and episomal targets up to 20 kb.

Description:

- onHybrid 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 10 min initial denaturation step.
- Use of onHybrid DNA Polymerase allows for the increase of PCR specificity, sensitivity and yield in comparison to the conventional PCR assembly method.
- onHybrid 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.
- onHybrid DNA Polymerase has enhanced target length capability with regard to genomic targets (up to 10 kb from human genomic DNA).
- **Due to the polymerase modification, the optimal reaction conditions (especially annealing temperatures) differ from standard PCR protocols**.
- onHybrid 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 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
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
onHybrid 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 the reaction buffer, because it may form a precipitate when frozen.
2. Prepare reaction mixes at room temperature. Use of onHybrid DNA Polymerase allows room temperature reaction setup. Mix well.
3. Reactions can be placed in a room temperature thermal cycler.
4. onHybrid DNA Polymerase should be the last component added to the PCR mixture. In the absence of dNTPs proofreading activity of onHybrid DNA Polymerase may degrade primers.
5. For onHybrid 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 onHybrid 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 onHybrid 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

Step	2-step protocol		3-step protocol		Number of Cycles
	Temp.	Time	Temp.	Time	
Initial Denaturation	98°C	10 min	98°C	10 min	1
Denaturation	98°C	5-10 s	98°C	5-10 s	25-35
Annealing	-	-	X°C	15-30 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

Notes:

1. onHybrid is activated by a 10-minute initial denaturation step at 98°C.
2. onHybrid 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 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 72°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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