

cat. # 1003-200



200 U



MARATHON DNA polymerase



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MARATHON DNA polymerase

- The product is a mixture of thermostable DNA polymerases *Taq* i *Pwo* supplemented by thermostable UTPase.
- Dedicated for amplification of long PCR products ranging 2-40 kb.
- The highest purity grade.

The Marathon DNA polymerase is a mixture of *Taq* and *Pwo* DNA polymerases supplemented by thermostable UTPase activity. Such an enzyme composition enables the efficient synthesis of long amplicons.

The *Taq* and *Pwo* DNA polymerases form mutually supporting duet during the DNA elongation process while UTPase removes deoxyUTP from reaction mixture. The deoxyUTP is considered as a major inhibitor of *Pwo* DNA polymerase activity.

Contents:

200 U Marathon DNA polymerase:
concentration: 1 U/ μ l

4 x 1,5 ml 2x PCR reaction buffer:
40 mM $(\text{NH}_4)_2\text{SO}_4$, 100 mM Tris pH 9.0, 5 mM MgCl_2 , 10% DMSO.

store at -20 °C

For R&D use only

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Marathon DNA polymerase - user's guidelines:

1. Use the thin wall PCR tubes only.
2. Always overlay your PCR mixtures with RNase and DNase free mineral oil (apply 50 μ l of oil for 50 μ l of reaction volume), even if your thermocycler is equipped with heating cover.
3. Use 1 to 5 °C lower annealing temperature comparing to the primers theoretical calculated temperatures.
4. Design your PCR primers for the lowest possible annealing temperature (range of 60-68 °C).
5. Use 200 ng of each primer for 50 μ l of PCR mixture. Primers longer than 23 nt have to be HPLC purified.
6. Use the highest grade of dNTPs for PCR at a concentration of 200-250 μ M of each dNTP (we recommend 10 mM dNTPs Mix 10, cat # 2001-200, 2001-1000).
7. Use the pure, high molecular weight DNA template at a concentration range of 250-1000 ng per 50 μ l of PCR volume (we recommend Genomic Midi AX kit, cat. # 895-20).
8. Store the DNA template at 4 °C.
9. Calculate carefully your long PCR thermal profile. Apply 1 min of elongation for each 1000 bp length of PCR product to be amplified.
10. The PCR amplification of DNA fragments exceeding the 20 kb may require two times higher concentration the Marathon DNA polymerase.

Exemplary PCR protocol:
initial denaturation: 93 °C - 60 s
DNA amplification: 30-40 cycles:
93 °C - 30 s, T_a -1 do 5 °C - 30 s, 68 °C - 60 s / 1 kb
final inubation: 10 °C

11. Obtained PCR products store in refrigerator or freezer.

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