NEBNext® High-Fidelity 2X PCR Master Mix

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M0541S 002120714011

M0541S

50 reactions (1.25 ml) **Lot: 0021207**

RECOMBINANT Store at –20°C Exp: 1/14

Description: The NEBNext High-Fidelity 2X PCR Master Mix is specifically optimized for robust, high-fidelity amplification of next-generation sequencing (NGS) libraries, regardless of GC content. The polymerase component of the master mix, Q5™ High-Fidelity DNA Polymerase, is a novel thermostable DNA polymerase that possesses 3´→5´ exonuclease activity, and is fused to a processivity-enhancing Sso7d domain. Q5 High-Fidelity DNA Polymerase also has an ultra-low error rate (> 50-fold lower than that of *Taq* DNA Polymerase and 6-fold lower than that of *Pyrococcus furiosus (Pfu)* DNA Polymerase). The buffer component of the master mix has been optimized for robust amplification, even with GC-rich amplicons. This combination makes the NEBNext High-Fidelity 2X PCR Master Mix ideal for NGS library construction.

This convenient 2X master mix contains dNTPs, Mg^{++} and a proprietary buffer, and requires only the addition of primers and DNA template for robust amplification. When used at the recommended 1X final concentration, the NEBNext High-Fidelity Master Mix contains 2 mM Mg^{++}.

Please Note: To ensure optimal performance, the master mix should be thawed and resuspended prior to use. Stability testing using up to 20 freeze/thaw cycles has shown no negative effect on master mix performance. The NEBNext High-Fidelity 2X PCR Master Mix may be liquid at –20°C.

Source: An *E. coli* strain that carries the Q5 High-Fidelity DNA Polymerase gene.

Application:

- Next generation sequencing library construction
- High fidelity PCR
- Difficult amplification
- High-throughput PCR

Reaction Conditions: NEBNext High-Fidelity 2X PCR Master Mix, DNA template and 0.5 µM to 1.25 µM primers (depending on sample input) in a total reaction volume of 50 µl.

Heat Inactivation: No

Quality Control Assays

16-Hour Incubation: A 50 µl reactions containing NEBNext High-Fidelity 2X PCR Master Mix and 1 µg of HindIII digested λ DNA incubated for 16 hours at 37°C results in no detectable non-specific nuclease degradation as determined by agarose gel electrophoresis. 50 µl reactions containing NEBNext High-Fidelity 2X PCR Master Mix and 1 µg of T3 DNA incubated for 16 hours at 37°C results in no detectable non-specific nuclease degradation as determined by agarose gel electrophoresis.

Phosphatase Activity: Incubation of NEBNext High-Fidelity 2X PCR Master Mix in protein phosphatase assay buffer (1 M diethanolamine @ pH 9.8 and 0.5 mM $MgCl_2$) containing 2.5 mM p-nitrophenyl phosphate at 37°C for 4 hours yields no detectable p-nitrophenylene anion as determined by spectrophotometric analysis at 405 nm.

Functional Activity (PCR): 30 cycles of PCR amplification of 20 ng genomic DNA in a 50 µl reaction containing 0.5 µM primers and 1X NEBNext High-Fidelity PCR Master Mix result in the expected 737 bp product.

PCR

Please note that protocols with NEBNext High-Fidelity 2X PCR Master Mix may differ from protocols with other polymerases. Conditions recommended below should be used for optimal performance.

Reaction Setup:
We recommend assembling all reaction components on ice and quickly transferring the reactions to a thermocycler preheated to the denaturation temperature (98°C). All components should be mixed prior to use.

COMPONENT	DNA PROTOCOL (1 µg-5 µg)	mRNA PROTOCOL (50 ng-250 ng PURIFIED mRNA)	ChIP DNA PROTOCOL 10 ng
NEBNext High-Fidelity 2X PCR Master Mix	25 µl	25 µl	25 µl
25 µM Primer	2.5 µl	1 µl	1 µl
25 µM Primer	2.5 µl	1 µl	1 µl
Adaptor-ligated DNA	20 µl	23 µl	23 µl

Notes: Gently mix the reaction. Collect all liquid at the bottom of the tube by a quick spin if necessary.

Transfer PCR tubes to a PCR machine and begin thermocycling.

Thermocycling Conditions for a Routine PCR:

STEP	TEMP	TIME	CYCLES
Initial Denaturation	98°C	30 seconds	1
Denaturation	98°C	10 seconds	4-15 Cycles (depending on starting material)
Annealing	65°C*	30 seconds	
Extension	72°C	30 seconds	
Final Extension	72°C	5 minutes	1
Hold	4°C		

*65°C is optimal for Illumina sample preparation. Depending on primer design, annealing temperature may need to be optimized.

General Guidelines:

1. Template:
   Use of high quality, purified DNA templates greatly enhances the success of PCR.

STARTING MATERIAL	AMOUNT	CYCLES
DNA	1 µg–5 µg	4–8
Purified mRNA	50–250 ng	10–12
ChIP DNA	10 ng	15

2. Mg^{++} and additives:
   The NEBNext High-Fidelity 2X PCR Master Mix contains 2.0 mM Mg^{++} when used at a 1X concentration. This is optimal for most PCR products generated with this master mix.
3. Deoxynucleotides:
   The final concentration of dNTPs is 200 µM of each deoxynucleotide in the NEBNext High-Fidelity 2X PCR Master Mix. Q5 High-Fidelity DNA Polymerase cannot incorporate dUTP and is not recommended for use with uracil-containing primers or templates.
4. DNA polymerase concentration:
   The concentration of DNA Polymerase in the NEBNext High-Fidelity 2X PCR Master Mix has been optimized for best results under a wide range of conditions.
5. Denaturation:
   An initial denaturation of 30 seconds at 98°C is sufficient for most sample types.

   During thermocycling, the denaturation step should be kept to a minimum. Typically, a 10 second denaturation at 98°C is recommended for most templates.
6. Annealing:
   Optimal annealing temperatures for NEBNext High Fidelity 2X PCR Master Mix tend to be higher than for other PCR polymerases. Depending on primer design, the annealing temperature may need to be optimized.
7. Extension:
   The recommended extension temperature is 72°C. Extension times are generally 30 seconds for libraries up to 1kb. Larger insert lengths may require additional time.

   A final extension of 5 minutes at 72°C is recommended.
8. Cycle number:
   Generally, 4–15 cycles yield sufficient product.
9. PCR product:
   The PCR products generated using NEBNext High-Fidelity 2X PCR Master Mix have blunt ends.
10. Perform clean up after the PCR reaction using SPRI beads or PCR purification columns.

(see other side)

Companion Products Sold Separately:

NEBNext DNA Library Prep Reagent Set for Illumina
#E6000S 12 reactions
#E6000L 60 reactions

NEBNext DNA Library Prep Master Mix Set for Illumina
#E6040S 12 reactions
#E6040L 60 reactions

NEBNext mRNA Library Prep Reagent Set for Illumina
#E6100S 12 reactions
#E6100L 60 reactions

NEBNext mRNA Library Prep Master Mix Set for Illumina
#E6110S 12 reactions
#E6110L 60 reactions

NEBNext ChIP-Seq Library Prep Reagent Set for Illumina
#E6200S 12 reactions
#E6200L 60 reactions

NEBNext ChIP-Seq Library Prep Master Mix Set for Illumina
#E6240S 12 reactions
#E6240L 60 reactions

NEBNext Singleplex Oligos for Illumina
#E7350S 12 reactions
#E7350L 60 reactions

NEBNext Multiplex Oligos for Illumina (Index Primers Set 1)
#E7335S 24 reactions
#E7335L 96 reactions

NEBNext Multiplex Oligos for Illumina (Index Primers Set 2)
#E7500S 24 reactions
#E7500L 96 reactions