NEBNext® Q5® Hot Start HiFi PCR Master Mix

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M0543S 004150817081

M0543S

50 reactions (1.25 ml) **Lot: 0041508**

RECOMBINANT Store at –20°C Exp: 8/17

Description: The NEBNext Q5 Hot Start HiFi 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 also has an ultra-low error rate (> 100-fold lower than that of *Taq* DNA Polymerase and ~12-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 and offers enhanced compatibility with a variety of beads used in typical NGS workflows. These features make the NEBNext Q5 Hot Start HiFi 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. The inclusion of the hot start aptamer allows convenient room temperature reaction set up.

Please Note: A precipitate can form upon thawing. To ensure optimal performance, the master mix should be thawed and resuspended prior to use. Stability testing using up to 30 freeze/thaw cycles has shown no negative effect on master mix performance. The NEBNext Q5 Hot Start HiFi PCR Master Mix may be liquid at –20°C.

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

Application:

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

Reaction Conditions: NEBNext Q5 Hot Start HiFi 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 reaction containing NEBNext Q5 Hot Start HiFi 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 Q5 Hot Start HiFi 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 Q5 Hot Start HiFi 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 (Multiplex PCR, Bead Inhibition): 30 cycles of PCR amplification of 20 ng genomic DNA with and without carboxylated magnetic beads in a 50 µl reaction containing 0.5 µM 4-plex primer mix and 1X NEBNext Q5 Hot Start HiFi PCR Master Mix result in the four expected amplicons and no inhibition of amplification in the presence of the beads.

PCR

Please note that protocols with NEBNext Q5 Hot Start HiFi PCR Master Mix may differ from protocols with other polymerases. Conditions recommended below should be used for optimal performance.

Reaction Setup:
NEBNext Q5 Hot Start HiFi PCR Master Mix is inhibited at room temperature, allowing flexible reaction setup (RT or ice). All components should be mixed prior to use.

COMPONENT	DNA PROTOCOL 1 µg-5 µg	DNA PROTOCOL 5 ng-1 µg	mRNA PROTOCOL 50 ng-250 ng PURIFIED mRNA	mRNA PROTOCOL TOTAL RNA 10 ng-1 µg	ChIP DNA PROTOCOL 10 ng
NEBNext Q5 Hot Start HiFi PCR Master Mix	*25 µl*	*25 µl*	*25 µl*	*25 µl*	*25 µl*
10 µM Primer	*5 µl*	*5 µl*	*2.5 µl*	*2.5 µl*	*2.5 µl*
10 µM Primer	*5 µl*	*5 µl*	*2.5 µl*	*2.5 µl*	*2.5 µl*
Adaptor-ligated DNA	*15 µl*	*15 µl*	*20 µl*	*20 µl*	*20 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*	*2-15 Cycles (depending on starting material)*
Annealing/ Extension	*65°C**	*75 seconds*	
Final Extension	*65°C*	*5 minutes*	*1*
Hold	*4–10°C*		

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

General Guidelines:

1. Use of high quality, purified DNA templates greatly enhances the success of PCR reactions. Recommended amounts of DNA template for a 50 µl reaction are as follows:

STARTING MATERIAL	PRODUCT #	AMOUNT	CYCLES
DNA	*E6000 and E6040*	*1 µg–5 µg*	*2–4*
	E7370	*5 ng–1 µg*	*4–12*
Purified mRNA	*E6100 and E6110*	*50–250 ng*	*10–12*
Total RNA	*E7420*	*100 ng–1 µg*	*12–15*
	E7530	*10 ng–1 µg*	*12–15*
ChIP DNA	*E6200 and E6240*	*10 ng*	*15*

2. Mg^{++} and additives:
The NEBNext Q5 Hot Start HiFi 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.

(see other side)

CERTIFICATE OF ANALYSIS

3. Deoxynucleotides:
The final concentration of dNTPs is 200 µM of each deoxynucleotide. 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 Q5 Hot Start HiFi 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 Q5 High-Fidelity DNA Polymerase 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 65°C. Extension times are generally 30 seconds for libraries up to 1 kb. Larger insert lengths may require additional time.

   A final extension of 5 minutes at 65°C is recommended.

8. Cycle number:
Generally, 4–12 cycles yield sufficient product.

9. PCR product:
The PCR products generated using NEBNext Q5 Hot Start HiFi PCR Master Mix have blunt ends.

10. Bead Compatibility:
The NEBNext Q5 Hot Start HiFi PCR Master Mix is compatible with a variety of carboxylated, tosylated and streptavidin beads that may be carried over or included in the PCR step of library construction protocols including Agencourt® AMPure® XP (Beckman Coulter, Inc.), Sera-Mag SpeedBeads and Mag-Bind® RXNPure Plus (Omega Bio-tek, Inc.). SPRI Beads or PCR purification columns are recommended for post PCR clean up.

Companion Products Sold Separately:

NEBNext Ultra DNA Library Prep Kit for Illumina
#E7370S 24 reactions
#E7370L 96 reactions

NEBNext Ultra Directional RNA Library Prep Kit for Illumina
#E7420S 24 reactions
#E7420L 96 reactions

NEBNext Ultra RNA Library Prep Kit for Illumina
#E7530S 24 reactions
#E7530L 96 reactions

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

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

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

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

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