NEBNext® Ultra™ II Q5® Master Mix

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M0543S 002140816082

M0544S

50 reactions (50 µl vol) **Lot: 0011508**

RECOMBINANT Store at –20°C Exp: 02/17

Description: The NEBNext Ultra II Q5 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 Ultra II Q5 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.

Stability: Stability testing using up to 30 freeze/thaw cycles has shown no negative effect on master mix performance. The NEBNext Ultra II Q5 Master Mix may be stored at –20°C for up to 18 months.

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 Ultra II Q5 Master Mix, DNA template and 1 µM primers in a total reaction volume of 50 µl.

Heat Inactivation: No

Quality Control Assays

16-Hour Incubation: A 50 µl reaction containing 25 µl of NEBNext Ultra II Q5 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. A 50 µl reaction containing 25 µl of NEBNext Ultra II Q5 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 Ultra II Q5 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 Ultra II Q5 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 Ultra II Q5 Master Mix may differ from protocols with other polymerases. Conditions recommended below should be used for optimal performance.

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

COMPONENT	VOLUME PER 50 µl RXN	FINAL CONCENTRATION
NEBNext Ultra II Q5 Master Mix	25 µl	1X
Primer pair	variable	1 µM
Adaptor-ligated DNA*	15 µl	variable
Nuclease-free water	to 50 µl	N/A

*From NEBNext DNA Library Prep Kit E6000, E6040, E7370 and E7645.

Transfer PCR tubes to a PCR machine and begin thermocycling.

Recommended Thermocycling Conditions:

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		

* The PCR conditions have been optimized for NEBNext Adaptor-ligated DNAs with NEBNext primer sets. For user-supplied adaptors and primers, a three-step PCR with a 45 second extension may be necessary depending on the optimal annealing temperature for your primer.

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:

PRODUCT #	INPUT DNA AMOUNT	PCR CYCLES*
E6000 & E6040	1 µg–5 µg	2–4
E7370	5 ng–1 µg	4–12
E7645	0.5 ng–1 µg	3–15

* Please refer to the specific product manual for detailed recommended cycle numbers tailored to your DNA input.

2. Mg^{++} and additives:
The NEBNext Ultra II Q5 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 optimized for robust library amplification. 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 Ultra II Q5 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.

(see other side)

CERTIFICATE OF ANALYSIS

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 for library amplification 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, 2–13 cycles yield sufficient product depending on the DNA input and specific DNA library prep product utilized. Please refer to the product manual for detailed cycle number recommendations

9. PCR product:
   The PCR products generated using NEBNext Ultra II Q5 Master Mix have blunt ends.

10. Bead Compatibility:
    The NEBNext Ultra II Q5 Master Mix is compatible with a variety of carboxylated and tosylated beads that may be carried over or included in the PCR step of library construction protocols including streptavidin beads, 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 II DNA Library Prep Kit for Illumina
#E7645S 24 reactions
#E7645L 96 reactions

NEBNext Ultra II End Repair/dA-tailing Module
#E7546S 24 reactions
#E7546L 96 reactions

NEBNext Ultra II Ligation Module
#E7595S 24 reactions
#E7595L 96 reactions

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

NEBNext DNA Library Prep Master Mix Set for Illumina
#E6040S 12 reactions
#E6040L 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 Multiplex Oligos for Illumina (Dual Index Primers Set 1)
#E7600S 96 reactions

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

NEBNext Library Quant Kit for Illumina
#E7630S 100 reactions
#E7630L 500 reactions