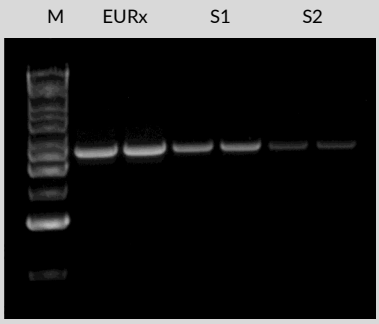
EURx® MOLECULAR BIOLOGY PRODUCTS

NXT tiTaq PCR Kit

Cat. No.	size
E2535-01	100 reactions of 20 µl
E2535-02	200 reactions of 20 µl
E2535-03	500 reactions of 20 µl

Storage Conditions: Store at -20°C.



Fast PCR amplification using EURx NXT tiTaq PCR Kit. A 2.5 kb amplicon of the human CCR5 gene was amplified with NXT tiTaq PCR Kit and fast-cycling PCR kits from two another suppliers.

Lane M: molecular size marker- Perfect 1 kb DNA Ladder.

Lanes 1, 2: PCR amplification reactions using EURx NXT tiTaq PCR Kit.

Lanes 3, 4: PCR amplification reactions using a fast-cycling PCR kit from Supplier 1.

Lanes 5, 6: PCR amplification reactions using a fast-cycling PCR kit from Supplier 2.

PCR program:

95°C 5 min

96°C 5 s

62°C 5 s

68°C 1 min 15 s

x35

72°C 1 min

Description:

- NXT tiTaq PCR Kit is designed for fast-cycling PCR on any thermal cycler.
- NXT tiTaq PCR Kit shortens PCR cycling time without affecting the yield and PCR performance.
- The annealing and extension steps require 5 s and 3 s per 100 bp respectively.
- PCR does not require redesigning of primers.
- NXT tiTaq PCR Kit is a ready-to-use solution containing hot start NXT tiTaq DNA Polymerase, reaction buffer, $MgCl_2$ and dNTPs.
- NXT tiTaq DNA Polymerase contains a new generation „hot start" enzyme that is blocked at moderate temperatures and allows room temperature reactions setup.
- The polymerase activity is restored during normal cycling conditions.
- "Hot start" PCR may increase specificity, sensitivity and yield of a PCR reaction in comparison to the conventional PCR assembly method.
- NXT tiTaq DNA Polymerase replicates DNA at 72°C and exhibits a half-life of 40 min at 95°C.
- Contains the 5'→3' exonuclease activity.
- Lacks the 3'→5' exonuclease activity.
- Adds extra A at the 3' ends.
- NXT tiTaq PCR Kit is supplied with 10 x Color Load solution which allows for direct loading PCR reactions on the gel.
- NXT tiTaq PCR Kit allows to obtain a wide range of PCR products up to 4 kb and 10 kb from complex genomic or episomal DNA respectively.

NXT tiTaq PCR Kit contains:

- NXT tiTaq PCR Master Mix (2x)
- Water, nuclease free
- 10 x Color Load

NXT tiTaq PCR Master Mix (2x):

NXT tiTaq PCR Master Mix (2x) contains NXT tiTaq DNA Polymerase, optimized reaction buffer, $MgCl_2$ and dNTPs.

10 x Color Load:

10 x Color Load contains two gel tracking dyes and a gel loading reagent. It enables direct loading of PCR products onto an agarose gel.

Quality Control:

All preparations are assayed for contaminating endonuclease, 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
NXT tiTaq PCR Master Mix (2x)	10 µl	1 x
Upstream primer	Variable	0.5 µM
Downstream primer	Variable	0.5 µM
Optional: 10 x Color Load	2 µl	1 x
Template DNA	Variable	<0.2 µg/20 µl
Sterile double-distilled water	To 20 µl	-
Total volume	20 µl	-

Notes:

- Do not use reaction volumes larger than 20 µl as this will interfere with the optimal temperature gradient required for successful results.
- Thaw, gently vortex and briefly centrifuge NXT tiTaq PCR Master Mix (2x), primers, DNA template before use to avoid localized differences in salt concentration.
- Set up PCR reactions at room temperature.
- Primers can be added separately or as a primer mix prepared previously.
- Vortex the samples and briefly spin down.
- Reactions can be placed in a room temperature thermal cycler.
- Use of 10 x Color Load allows PCR reactions to be loaded directly on the gel without prior addition of a gel loading buffer. 10 x Color Load contains a gel loading reagent and two tracking dyes (a red dye and a yellow dye) that separate during electrophoresis. The red dye migrates at the same rate as 600 bp DNA fragment and the yellow dye migrates faster than 20 bp in a 1% agarose gel. The dyes do not interfere with most downstream enzymatic applications, however it is recommended to purify PCR products prior enzymatic manipulation.
- In most cases there is no need to add additives to the PCR reaction. For some difficult targets such as: GC-rich sequences, sequences with complex secondary structures additives such as DMSO can be added to improve amplification. Use DMSO in concentrations of 2-8%. The recommended starting DMSO concentration (if needed) is 3%.
- As a general guide for how much template DNA to use, start with a minimum 10^4 copies of the target sequence to obtain a signal in 25-35 cycles (i.e. 1 µg of 1 kb ds DNA equals 9.1 x 10^{11} molecules, 1 µg of *E. coli* genomic DNA equals 2 x 10^8 molecules, 1 µg of human genomic DNA equals 3 x 10^5 molecules).

Thermal Cycling Conditions:

Step	Temperature	Time	Number of Cycles
Initial Denaturation	95°C	2-5 min	1
Denaturation	96°C	5 s	25-40
Annealing	50-68°C	5 s	
Extension	68°C	3 s/100 bp	
Final Extension	72°C	1 min	1
Cooling	2-8°C	Indefinite	1

Notes:

1. For comlex genomic DNA and GC-rich templates 5-min initial denaturation is strongly recommended.
2. Annealing temperature should be optimized for each primer set based on the primer T_m. Optimal annealing temperatures may be above or below the estimated T_m. As a starting point, use an annealing temperature 5°C below T_m.

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