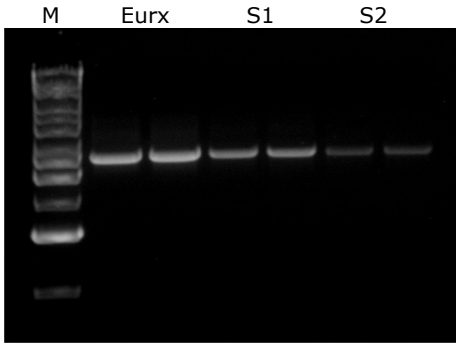
NXT Taq PCR Kit

Cat. No.	Size
E2530-01	100 reactions of 20 µl
E2530-02	200 reactions of 20 µl
E2530-03	500 reactions of 20 µl

Storage Conditions: Store at -20°C.



Fast PCR amplification using EURx NXT Taq PCR Kit. A 2.5 kb amplicon of the human CCR5 gene was amplified with NXT Taq 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 Next Taq 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 5s
60°C 5s
68°C 1 min 15 s
x31
72°C 1 min

Description:

- NXT Taq PCR Kit is designed for fast-cycling PCR on any thermal cycler.
- NXT Taq 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 100bp respectively.
- PCR does not require redesigning of primers.
- Next Taq PCR Kit is a ready-to-use solution containing hot start NXT Taq DNA Polymerase, reaction buffer, $MgCl_2$ and dNTPs.
- Anti-Taq antibodies inhibit polymerase activity at moderate temperature.
- The polymerase activity is restored during the initial denaturation step.
- Formation of complexes between Taq DNA Polymerase and an anti-Taq antibody forms a basis for "hot start" PCR, which allows for convenient room-temperature reaction setup.
- "Hot start" PCR may increase specificity, sensitivity and yield of a PCR reaction in comparison to the conventional PCR assembly method.
- NXT Taq 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.
- Next Taq PCR Kit is supplied with 10 x Color Load solution which allows for direct loading PCR reactions on the gel.
- Next Taq PCR Kit allows to obtain a wide range of PCR products up to 4 kb and 10 kb from episomal or complex genomic DNA respectively.

NXT Taq PCR Kit contains:
1. NXT Taq PCR Master Mix (2x)
2. Water, nuclease free
3. 10 x Color Load

NXT Taq PCR Master Mix (2x):
NXT Taq PCR Master Mix (2x) contains NXT Taq 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 on the gel.

Preparation of PCR Reaction:

Component	Volume/reaction	Final concentration
NXT Taq 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
Water, nuclease free	To 20 µl	-
Total volume	20 µl	-

Notes:

1. Do not use reaction volumes larger than 20 µl as this will interfere with the optimal temperature gradient required for successful results.
2. Thaw, gently vortex and briefly centrifuge NXT Taq PCR Master Mix (2x), primers, DNA template before use to avoid localized differences in salt concentration.
3. Set up PCR reactions at room temperature.
4. Primers can be added separately or as a primer mix prepared previously.
5. Vortex the samples and briefly spin down.
6. Reactions can be placed in a room temperature thermal cycler.
7. 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 an 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.
8. 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%.
9. 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:

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

Notes:

1. 2-5 min initial denaturation step at 95°C is required to inactivate the antibody, restore the polymerase activity and denaturate the template.
2. For comlex genomic DNA and GC-rich templates 5-min denaturation is strongly recommended.
3. 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.