MANUFACTURED BY EURx

roboklon

NXT *Taq* - *FAST* PCR KIT HOT START

High Speed, Next Gen Taq PCR Master Mix

Taq DNA Polymerase

(Thermus aquaticus)
- modified -

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

Storage Conditions: Store at -20°C for long-term storage (more than 12 months) or at 4°C for up to 2 months.

NXT *Taq* DNA Polymerase Properties:

Feature	Value	Remarks
Template nucleic acids	dsDNA ssDNA cDNA	
5'-3' exonuclease	+	
3'-5' exonuclease	-	none
Proofreading	-	no
Strand displacement	-	weak
Primase	-	
Relative accuracy (plain *Taq* = 1)	1	
Amplicon length	To 10 kb	
Extension velocity	~35 bp/s 2 kb/min	
Addition of extra 3'-A overhangs	yes	
TA- / blunt cloning possible	yes / yes	
Temperature range (elongation step)	62°C - 72°C	Optimum: 68°C; low G+C: <68°
Half life at 95°C	40 min	
Recommended PCR applications	Fast PCR HotStart PCR Multiplex PCR DNA fingerprinting Genotyping Generic PCR	

* Relative accuracy ratio := Error rates NXT *Taq* / *Taq*. A value of 10 would indicate 10-fold higher accuracy as compared to plain *Taq* DNA Polymerase, a value of 1 indicates similar accuracy / precision.

Enables complete PCR runs within dramatically shortened amplification times.

Description:

- NXT *Taq* PCR Kit is a dedicated fast-cycling PCR kit, showing robust performance and reliable high-yield amplification on any thermal cycler.
- NXT *Taq* PCR Kit dramatically shortens PCR cycling time (depending on amplicon length, within less than 45-60 min total run time for most PCR reactions) while maintaining high yield and optimized performance.
- Overall annealing time is reduced to only 5 sec. DNA extension requires just 3 sec per 100 bp, even with difficult template DNA.
- PCR with fast NXT *Taq* does not require any redesign of primers. Existing PCR conditions can be adopted, while annealing and extension time can be greatly shortened.
- NXT *Taq* PCR Kit is supplied as a ready-to-use master mix, 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* PCR Kit is supplied with 10x Color Load solution which allows for direct loading of PCR reactions to agarose gels.
- The NXT *Taq* PCR Kit allows to obtain a wide range of PCR products up to 10 kb in length.

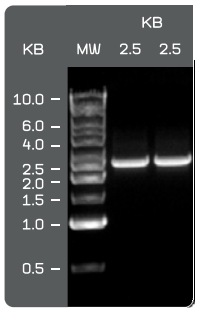

Figure 1: Fast PCR amplification using EURx NXT Taq PCR Kit. A 2.5 kb amplicon of the human CCR5 gene was amplified with NXT Taq PCR Kit and the following PCR conditions: 95°C 5 min - 31x (96°C 5s - 60°C 5s - 68°C 1 min 15 s) - 72°C 1 min
Lane M: molecular size marker- Perfect 1 kb DNA Ladder.
Lanes 1,2: PCR amplification reactions using NXT Taq PCR Kit (Cat. No.E3130).

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 (HotStart), 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 agarose gels.

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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PCR PROTOCOL

Preparation of PCR Reaction:

Component	Volume / Reaction	Final Concentration
NXT *Taq* PCR Master Mix (2x)	10 µl	1 x
Upstream primer	Variable	0.1-0.5 µM
Downstream primer	Variable	0.1-0.5 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	-

General formula for calculating total gene / genome copy numbers from the total amount of template DNA:

$$Template\ DNA\ copy\ number\ [molecules] =$$

$$\frac{DNA\ amount\ [ng] \cdot 6.022 \times 10^{23}\ [molecules\ mol^{-1}]}{Genomic\ DNA\ length\ [kb] \cdot 616\ [g\ mol^{-1}\ bp^{-1}]} \cdot \frac{10^{-3}\ [kb\ bp^{-1}]}{10^{9}\ [ng\ g^{-1}]}$$

Optimum: 10^4 template DNA copies
Maximum: 0.5 µg template DNA or less

Notes:

1. **Concentration Differences:** Thaw, gently vortex and briefly centrifuge NXT *Taq* PCR Master Mix (2x), primers, DNA template before use to avoid localized differences in salt concentration.
2. **Room Temperature:** Set up PCR reactions at room temperature. Use of NXT *Taq* PCR Kit (HotStart) allows room temperature reaction setup.
3. **Primer Mix:** Primers can be added separately or, more convenient, as a previously prepared primer mix.
4. **Mix Template:** Vortex the samples and spin down briefly.
5. **No Preheating Required:** Reactions can be placed in a room temperature thermal cycler. Preheating of the PCR cycler is not required.
6. **Color Load:** Use of the 10 x Color Load allows PCR reactions to be loaded directly onto an agarose gel without prior addition of a gel loading buffer.The 10 x Color Load contains a gel loading reagent and two gel tracking dyes (a red dye and an yellow dye) that separate during electrophoresis. In a 1 % agarose gel, the red dye migrates at the same rate as 600 bp DNA fragment and the yellow dye migrates faster than 20 bp. The dyes do not interfere with most downstream enzymatic applications, however it is recommended to purify PCR products prior to enzymatic manipulation.
7. **PCR Enhancers:** In most cases there is no need to add any 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% [v/v]. The recommended starting DMSO concentration (if needed) is 3% [v/v].
8. **Template Copies:** 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 9.8 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	Tempera-ture	Time	Number of Cycles
Initial Denaturation	95°C	2-5 min	1
Denaturation	96°C	5 s	25-35
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. **Initial Denaturation:** A 2 min initial denaturation step at 94-95°C is required to inactivate the antibody and to restore polymerase activity.
2. **Denaturing Difficult Templates:** For complex genomic DNA as well as for GC-rich templates, respectively, a 5-min denaturation period is strongly recommended.
3. **Annealing:** 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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