Instructions for Use
Life Science Kits & Assays

innuTaq DNA Polymerase

analytikjena
An Endress+Hauser Company

Content

1 Product and order number

Name	Amount	Order-no.
innuTaq DNA Polymerase	500 U	845-EZ-1000500

2 Storage conditions

Store innuTaq DNA Polymerase at -22 to -18 °C in a freezer with constant temperature conditions.

If stored as recommended, the innuTaq DNA Polymerase will remain stable until the expiration date printed on the kit label.

3 Description

The innuTaq DNA Polymerase is a highly purified recombinant thermostable DNA polymerase that has been isolated from *E. coli* carrying a vector encoding the *Thermus aquaticus* DNA polymerase gene.

The enzyme has 5' → 3' DNA polymerase activity, low 5' → 3' exonuclease activity and lacks 3' → 5' exonuclease activity. It exhibits high thermal stability in withstanding prolonged incubations at elevated temperatures (95 °C).

It is supplied with 25 mM $MgCl_2$ Solution, 10x PCR Buffer with KCl and 10x PCR Buffer with $(NH_4)_2SO_4$.

3.1 Quality data

Activity and stability tested at 20, 30 and 40 cycles of PCR reactions at 95 °C. Presence of *E.coli* DNA is < 10 copies per unit Taq DNA Polymerase.

3.2 Unit definition

One unit of enzyme catalyzes the incorporation of 10 nmol of deoxyribonucleotides (dNTP's) into a polynucleotide fraction in 30 minutes at 70 °C.

4 Delivered components

4.1 innuTaq DNA Polymerase 

Concentration: 5 U/µl

Enzyme storage buffer

The enzyme is supplied in: 20 mM Tris-HCl pH 8.0, 100 mM KCl, 1 mM DTT, 0.1 mM EDTA, 0.5 % Tween 20, 50 % glycerol, 0.5 % Nonidet P40.

4.2 PCR buffers

The innuTaq DNA Polymerase (5 U/µl) is provided with two different 10x PCR Buffers:

10x PCR Buffer with KCl 

100 mM Tris-HCl (pH 8.8 at 25 °C), 500 mM KCl, 0.8 % Nonidet P40.

10x PCR Buffer with $(NH_4)_2SO_4$ 

750 mM Tris-HCl (pH 8.8 at 25 °C), 200 mM $(NH_4)_2SO_4$, 0.1 % Tween 20.

Both 10x PCR Buffers do not contain $MgCl_2$.

4.3 Mg^{2+} Solution 

The innuTaq DNA Polymerase (5 U/µl) is provided with a 25 mM $MgCl_2$ Solution.

5 PCR conditions

5.1 Concentrations for standard thermal cyclers

- Gently vortex and briefly centrifuge all solutions after thawing.
- Mix following components in a thin-walled PCR tube, on ice.

Component	Volume	Final conc.
PCR-grade H_2O	Variable (add to a final vol. of 50 µl)	
10x PCR Buffer	5 µl	1x
25 mM $MgCl_2$ Solution	3 - 5 µl	1.5 - 2.5 mM
12.5 mM dNTP Mix	1 µl	0.25 mM
Forward Primer	Variable	0.2 - 1 µM
Reverse Primer	Variable	0.2 - 1 µM
Template DNA	Variable	1 - 100 ng/µl (≤ 1 µg)
innuTaq DNA Polymerase (5 U/µl)	0.2 - 0.5 µl	1 - 2.5 U
Total volume	50 µl	

5.2 Recommended time and temperature protocol

Step	Cycle	Profile	Temperature	Time
1	1	Initial denaturation	95 °C	3 - 5 min
2	26 - 35	Denaturation	95 °C	30 - 60 sec
		Annealing	50 - 68 °C	30 - 60 sec
		Elongation	72 °C	1 - 4 min
3	1	Final elongation	72 °C	5 - 10 min

Note: Annealing temperature should be 2 - 6 ºC lower than melting temperature of primer.

5.3 Hints and Notes

- Gently vortex and briefly centrifuge all solutions after thawing
- After pipetting mix the components of the reaction mix by gently vortexing and briefly centrifuge for a few seconds to collect the mixture at the bottom of the tube.
- Keep the reaction tubes on ice as long as possible.
- Transfer samples from ice to the thermal cycler. **Note:** If a thermal cycler without Sample Protection System is used, wait until denaturation temperature of about 94 °C has been reached.

Reaction conditions (incubation temperatures and times, concentrations of template DNA, primers, magnesium ions and enzyme) are depending on the used template and primers.

The optimal Mg^{2+} concentrations vary between 1 - 4 mM and have to be determined empirically. However, many applications work at the standard concentration of 1.5 mM Mg^{2+}. Advanced applications on genomic DNA require higher Mg^{2+} concentrations (2 - 3 mM) adjustable by using the separate 25 mM $MgCl_2$ Solution supplied with the set.

6 Related products

Product	Amount	Order Number
50x inNucleotide Mix	2x 0.5 ml	845-AS-9000100
inNucleotide Set (100 mM)	4x 0.25 ml	845-AS-1100250
6x Loading Dye Orange G	6x 1.0 ml	845-ST-4010006
6x Loading Dye Bromophenol Blue	6x 1.0 ml	845-ST-3010006
innuSTAR 100 bp DNA Ladder Express	500 µl	845-ST-1010100
	5x 500 µl	845-ST-1010500
innuSTAR 1 kb DNA Ladder Express	500 µl	845-ST-1020100
	5x 500 µl	845-ST-1020500
innuMIX qPCR MasterMix Probe	1 ml (100 rxn)	845-AS-1200100
	2 ml (200 rxn)	845-AS-1200200
	10 ml (1000 rxn)	845-AS-1201000
innuMIX qPCR MasterMix SyGreen	1 ml (100 rxn)	845-AS-1300100
	2 ml (200 rxn)	845-AS-1300200
	5 ml (500 rxn)	845-AS-1300500

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