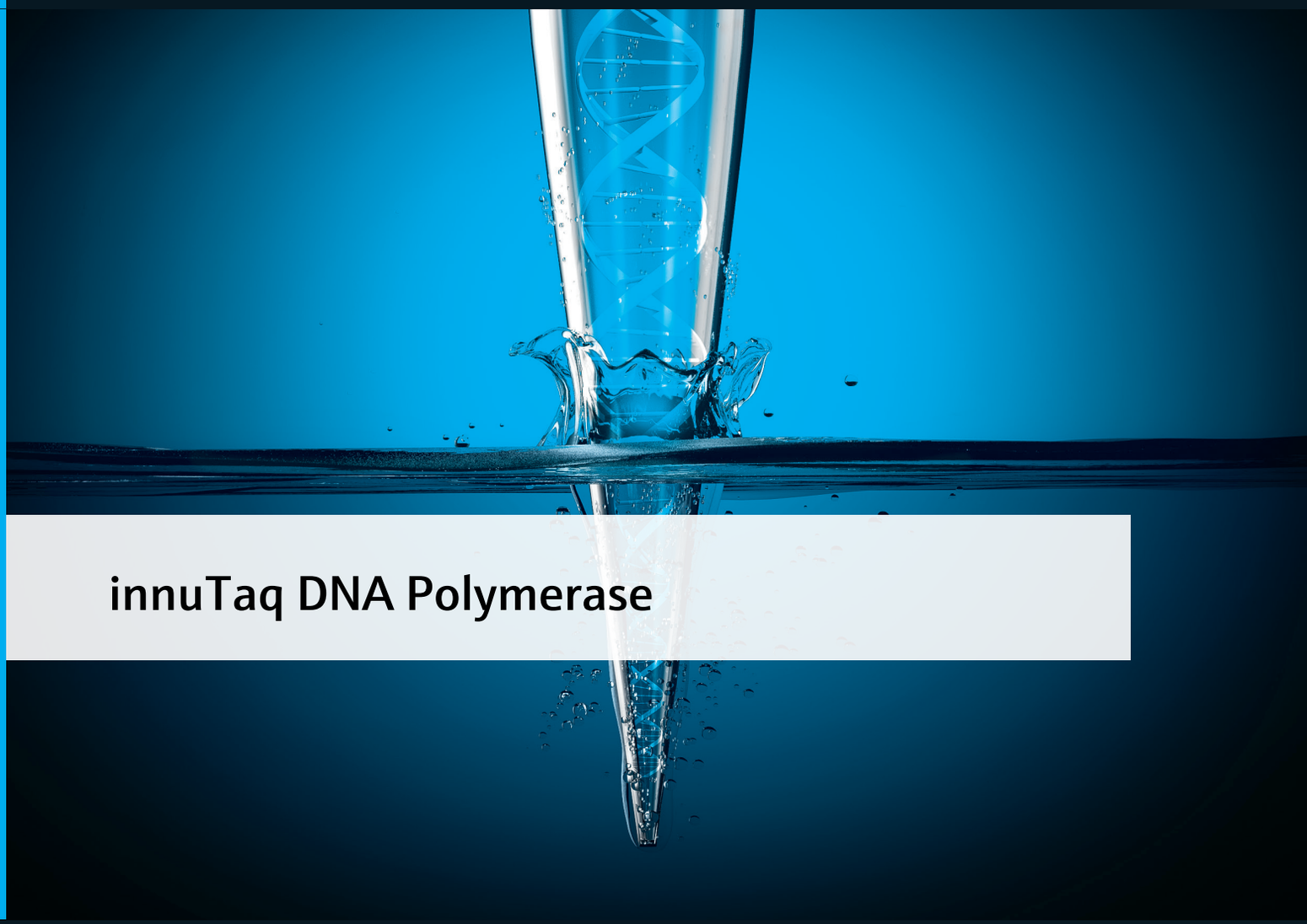
Instructions for Use
Life Science Kits & Assays



innuTaq DNA Polymerase

iST Innuscreen GmbH

1 Product specifications

The innuTaq DNA Polymerase is a highly purified, thermostable recombinant DNA polymerase. It 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 no 3′ → 5′ exonuclease activity. It exhibits high thermal stability in with prolonged incubations at elevated temperatures (95 °C).

Delivered components

Components		Description	Amount
innuTaq DNA Polymerase		Concentration: 5 U/µl Enzyme storage buffer contains 50 % glycerol	500 U
10x PCR Buffer With KCl		100 mM Tris-HCl, pH 8,8; 500 mM KCl; 0,8% Nonidet P40	1800 µl
10x PCR Buffer With $(NH_4)_2SO_4$		750 mM Tris, pH 8,8; 200 mM $(NH_4)_2SO_4$; 0,1% Tween 20	1800 µl
Mg^{2+} Solution		25 mM $MgCl_2$	1800 µl

2 Product and order number

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

3 Storage conditions

innuTaq DNA Polymerase is delivered at ambient temperature.

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

When stored as recommended, the innuTaq DNA Polymerase is stable until the expiration date printed on the label on the kit box.

4 Quality data and unit definition

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

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

5 Safety precautions

The assay shall only be handled by educated personal in a laboratory environment. The compliance with the specified procedure is absolutely mandatory when performing this assay.

Reagents should be stored in their original containers at the indicated temperatures. Do not replace individual components with those from different batches or test assays. Note the indicated expiration dates.

Do not eat, drink or smoke while performing the assay.

Wear protective clothing and safety gloves.

All samples and test materials should be handled and disposed of as infectious material, in accordance with regulatory requirements.

Reagent containers that have not come in contact with potentially infectious material may be disposed of along with ordinary laboratory waste.

Store the reagents used for performing PCR separately from DNA templates and amplification products.

6 Reagent preparation

- After thawing gently vortex and briefly centrifuge all solutions.
- Prepare on ice a mix of following components.

Reagent	Volume (1 rxn)
10x PCR Buffer (with or without $MgCl_2$)	5 µl
25 mM $MgCl_2$ Solution	3-5 µl
12.5 mM dNTP Mix	1 µl
Forward Primer	0.2 - 1 µM
Reverse Primer	0.2 - 1 µM
innuTaq DNA Polymerase (5 U/µl)	0.2 - 0.5 µl
Template DNA	1 - 100 ng/µl (≤ 1 µg)
PCR-grade H_2O	add to a final vol. of 50 µl
Total volume	50 µl

7 PCR conditions

Step	Cycles	Profile	Temperature	Retention time
1	1	Initial denaturation	95 °C	120 s
2	25-40	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 °C lower than melting temperature of primer.

8 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 before transferring samples to the thermal cycler.

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 50 mM $MgCl_2$ Solution supplied with the set.

9 Related products

Product	Order Number
50x inNucleotide Mix	845-AS-9000100
inNucleotide Set (100 mM)	845-AS-1100250

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