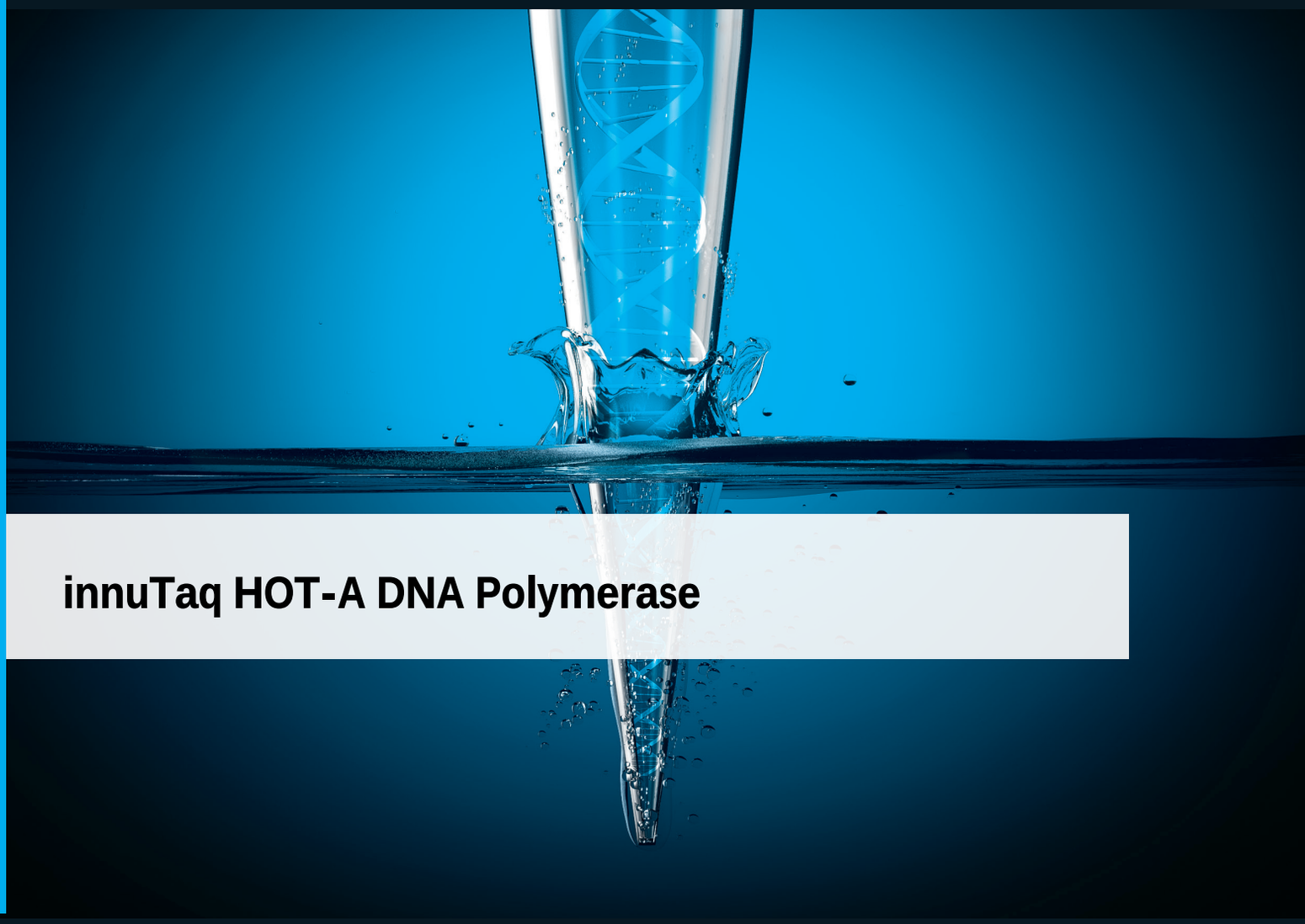
Instructions for Use
Life Science Kits & Assays



innuTaq HOT-A DNA Polymerase

Innuscreen GmbH

1 Product specifications

The innuTaq HOT-A DNA Polymerase provides improved specificity and sensitivity when amplifying low-copy-number targets in complex backgrounds. The polymerase activity is blocked at ambient temperature with a polymerase specific aptamere and switched on at the onset of the initial denaturation. The thermal activation prevents the extension of non specifically annealed primers and primer-dimer formation at low temperatures during PCR set-up.
It catalyses the polymerisation of nucleotides into duplex DNA in 5′ → 3′ direction in the presence of magnesium. It also possesses a 5′ → 3′ polymerisation – dependent exonuclease replacement activity but lacks a 3′ → 5′ exonuclease activity

Delivered components

Components		Description	Amount
innuTaq HOT-A DNA Polymerase		Concentration: 5 U/µl Enzyme storage buffer: 20 mM Tris-HCl, 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.5 % Tween 20, 0.5 % Nonidet P40, 50 % Glycerol, pH 8.0. l	500 U
10x Hot Start Buffer complete		200 mM Tris-HCl (pH 8.5), 500 mM KCl, 15 mM $MgCl_2$	1500 µl
10x Hot Start Buffer without $MgCl_2$		200 mM Tris-HCl (pH 8.5), 500 mM KCl	1500 µl
Mg2+ Solution		25 mM $MgCl_2$	1500 µl

2 Product and order number

Name	Amount	Order-no.
innuTaq HOT-A DNA Polymerase	500 U	845-EZ-3000500

3 Storage conditions

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

When stored as recommended, the innuTaq HOT-A 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 low copy PCR, human DNA contamination and activity of DNase and RNase are not detected. Polymerization activity at 25 ˚C is not detected.

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

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 a mix of following components.

Reagent	Volume (1 rxn)
10x Hot Start Buffer (complete or without $MgCl_2$)	5 µl
25 mM $MgCl_2$ Solution (if required)	3-5 µl
12.5 mM dNTP Mix	1 µl
Forward Primer	0.2 - 1 µM
Reverse Primer	0.2 - 1 µM
innuTaq HOT-A 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.
- The enzyme allows the PCR set up at ambient temperature without non-specific annealing and extension.

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 Mg2+ concentrations vary between 1 - 4 mM and have to be determined empirically. However, most applications work at the standard concentration of 1.5 mM Mg2+. Advanced applications on genomic DNA require higher Mg2+ concentrations (2 - 3 mM) adjustable by using the separate 25 mM $MgCl_2$ Solution supplied with the set.

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