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Manual

RUN-HS 5 DNA polymerase

Hot Start *Taq* DNA polymerase with the reaction buffer. Anti-Taq monoclonal antibody-based Hot Start technology. Concentration 5 U/µl.

catalog #	size
1001-200H-5	200 U
1001-1000H-5	1000 U

For research use only.

Guarantee

A&A Biotechnology provides guarantee on this product.
The company does not guarantee correct performance of this kit in the event of:

- not adhering to the supplied protocol
- use of not recommended equipment or materials
- use of other reagents than recommended or which are not a component of the product
- use of expired or improperly stored product or its components

Advantages

- *Taq* DNA polymerase is the most popular DNA polymerase in PCR procedures.
- The enzyme is recommended for routine PCR reactions.
- Hot start technology avoids nonspecific amplification and enables room temperature reaction setup.

Description

RUN-HS 5 DNA polymerase is *Taq* polymerase purified from *E. coli* strain carrying a plasmid with a cloned gene encoding a DNA polymerase from *Thermus aquaticus*. Enzyme catalyzes incorporation of deoxynucleotides to 3' end of dsDNA at temperature 70-80 °C and presence of Mg^{2+} ions.
Polymerase is blocked with the anti-Taq monoclonal antibody. Full activation time requires 5 min of incubation at 95 °C. *Taq* DNA polymerase lacks 3'-5' exonuclease activity, but possesses weak 5'-3' exonuclease activity.

Contents

	1001-200H-5	**1001-1000H-5**	**storage**
RUN-HS 5 polymerase storage buffer: 100 mM KCl, 50 mM Tris-HCl pH 8.5, 0.5% Tween, 0.5% Triton X-100, 50% glicerol (v/v).	200 U	1000 U	-20 °C
RUN-HS reaction buffer 10x PCR reaction buffer: 100 mM KCl, 100 mM $(NH_4)_2SO_4$, 200 mM Tris-HCl pH 8.5, 20 mM $MgSO_4$, 1% Igepal.	1 x 1.5 ml	4 x 1.5 ml	-20 °C

Unit definition

One unit of the enzyme catalyzes the incorporation of 15 nmol of dNTP into a polynucleotide fraction in 30 min at 75 °C.

Notes

- Before using, thoroughly thaw and gently mix by inverting the tubes.

PCR protocol

1. Add to the PCR tubes:

component	volume 50 µl	final concentration
RUN-HS reaction buffer	5 µl	1X
dNTP Mix (10 mM)	1-1.25 µl	200-250 µM
Primer 1 (10 µM)*	1 µl	0.2 µM
Primer 2 (10 µM)*	1 µl	0.2 µM
RUN-HS 5 polymerase	0.25 µl	1.25 U
DNA template	variable	10 pg -1 µg
ultrapure water	up to 50 µl	

*For optimization, a primer titration should be performed from 0,2 µM do 1 µM final concentration.

2. Gently mix the samples and briefly centrifuge.

3. Place the tubes in the thermocycler and start the PCR programme.
An example amplification profile:

reaction step	temperature	time	number of cycles
enzyme activation	95 °C	5 min	1
denaturation	95 °C	15 s	40
annealing*	50-68 °C	30 s	
extension**	72 °C	30 s	

*Annealing temperature depends on primer sequence and the composition of the reaction mixture.
**Time of extension depends on the length of the amplicon.

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