DATA SHEET

Jena Bioscience

Hot Start Polymerase Ab⁺ - glycerol-free

Heat-activatable DNA polymerase for high specificity, antibody-blocked in glycerol-free storage buffer
Thermus aquaticus, recombinant, *E. coli*

Cat. No.	Amount
PCR-424-1KU	1 kilo unit
PCR-424-10KU	10 kilo units
PCR-424-100KU	100 kilo units
PCR-424-1MU	1 Mio units

Unit Definition: One unit is defined as the amount of the enzyme required to catalyze the incorporation of 10 nmoles of dNTP's into an acid-insoluble form in 30 minutes at 70 °C using hering sperm DNA as substrate.

For general laboratory use.

Shipping: shipped on gel packs

Storage Conditions: store at -20 °C

Additional Storage Conditions: avoid freeze/thaw cycles

Shelf Life: 6 months

Form: liquid

Concentration: 5 units/µl

Description:
Hot Start Polymerase Ab⁺ - glycerol-free is recommended for use in freeze drying applications where glycerol must be avoided.
The polymerase provides improved specificity and sensitivity when amplifying low-copy-number targets in complex backgrounds or when prolonged room-temperature set up is required. The polymerase activity is blocked at ambient temperature and switched on automatically at the initial denaturation. The thermal activation prevents the extension of non-specifically annealed primers and primer-dimer formation at low temperatures during PCR setup. The polymerase is recommended for diagnostic applications, high throughput PCR or genotyping.
The enzyme replicates DNA at 72 °C. It catalyzes the polymerization of nucleotides into duplex DNA in 5'→3' direction in the presence of magnesium. It also possesses a 5'→3' polymerization-dependent exonuclease replacement activity but lacks a 3'→5' exonuclease (proof-reading) activity.

Activation step
Hot Start Polymerase Ab⁺ requires no prolonged heating or denaturing step. The polymerase inhibiting antibody is released within 2 min at 92°C during the initial denaturation step.

Content:
Reaction Buffer and Nucleotides are not included. Please refer to our our sections Buffers & Components and Nucleotides.

Hot Start Polymerase Ab⁺
5 units/µl Hot Start Polymerase Ab⁺ in 20 mM Tris-HCl, 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, stabilizer, cryo-protector, pH 9.0

Preparation of the master mix:
Before starting, thaw up all components and vortex thoroughly to ensure homogeneity.

component	final assay conc.
Reaction Buffer	1 x
dNTP Mix	200 µM
Hot Start Polymerase Ab⁺	0.025-0.05 units/µl
primer mix or each primer	200-400 nM each primer
PCR-grade Water	fill up to final volume

Cycling Conditions:

initial denaturation	95 °C	2 min	1 x
denaturation annealing [1] elongation [2]	95 °C 50-68 °C 72 °C	10-20 sec 10-20 sec 20 sec - 4 min	25-30 x

[1] The annealing temperature depends on the melting temperature of

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the primers used.
[2)] The elongation time depends on the length of the fragments to be amplified. A time of 1 min/kb is recommended.

Recommended Buffer Systems:
qPCR Buffer (#PCR-279) is recommended for use in real-time PCR applications. The buffer contains a well-balanced ratio of potassium-, ammonium- and magnesium-ions to ensure high specificity and minimal by-product formation in probe-based assays as well as in SybrGreen/EvaGreen-based assays. There is no need of additional optimization.,

KCl Buffer (#PCR-262) is recommended for use in routine PCR reactions. The buffer is optimized for highest specificity but may require additional fine-tuning of assay parameters like $MgCl_2$ concentration and annealing temperature.

Optimization of $MgCl_2$ concentration:
$MgCl_2$ Solution - 25 mM (#PCR-266) is recommended for optimization of the final Mg^{2+} concentration. A concentration of 1.5-2.0 mM is required for optimal functionality. Lower concentrations give higher specificity, whereas higher concentrations give higher yield.

Related Products:
dNTP Mix - 25 mM, #NU-1023
qPCR Buffer, #PCR-279
Crystal Buffer, #PCR-271
KCl Buffer, #PCR-262
$MgCl_2$ Solution - 25 mM, #PCR-266”