DATA SHEET

Jena Bioscience

# Hot Start Polymerase Ab+

Heat-activatable DNA polymerase for high specificity, antibody-blocked without buffer
Thermus aquaticus, recombinant, *E. coli*

| Cat. No. | Amount |
|---|---|
| PCR-423-1KU | 1 kilo unit |
| PCR-423-10KU | 10 kilo units |
| PCR-423-100KU | 100 kilo units |
| PCR-423-1MU | 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:** 12 months

**Form:** liquid

**Concentration:** 5 units/µl

**Description:**
Hot Start Polymerase Ab+ 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 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, stabilizers, 50 % (v/v) Glycerol, pH 9.0 (25°C)

**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<br>annealing [1]<br>elongation [2] | 95 °C<br>50-68 °C<br>72 °C | 10-20 sec<br>10-20 sec<br>20 sec - 4 min | 25-30 x |

[1] The annealing temperature depends on the melting temperature of the primers used.
[2] The elongation time depends on the length of the fragments to be

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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.,

*Crystal Buffer (#PCR-271)* is recommended for down-stream applications such as DNA sequencing, ligation, restriction digestion or where an analysis of the PCR product by absorbance or fluorescence excitation is required. For gel electrophoresis add gel loading buffer and fluorescent DNA stain (e.g. Gel Loading Buffer with DNA Stain, #PCR-274 - #PCR-276) before loading the PCR into the gel. Using pre-stained gels or post-run staining protocols is also possible.,

*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.,

*Ruby Buffer (#PCR-272)* includes a density reagent + tracking dye and allows the direct loading of the PCR products into an electrophoresis gel. For DNA detection / fluorescent DNA staining, we recommend to use new generations dyes (e.g. SYBR DNA Stain, #PCR-273) instead of the classical but highly mutagenic ethidium bromide.

**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
qPCRBuffer, #PCR-279
Ruby Buffer, #PCR-272
Crystal Buffer, #PCR-271
KCl Buffer, #PCR-262
$MgCl_2$ Solution - 25 mM, #PCR-266”