DATA SHEET

Jena Bioscience

Hot Start Core Kit Ab+

Kit of antibody-blocked hot start DNA polymerase, dNTPs and reaction buffer

Cat. No.	Amount
PCR-216S	200 units
PCR-216L	1.000 units
PCR-216XL	5.000 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 *in vitro* use only!

Shipping: shipped on blue ice

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 Core Kit Ab+ contains all reagents required for PCR (except template and primer) in one box combining simple handling with high flexibility.
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 nonspecifically annealed primers and primer-dimer formation at low temperatures during PCR setup. The polymerase is recommended for routine PCR applications (up to 4 kb fragment length), high throughput PCR or genotyping.
The Crystal Buffer system guarantees robust and reliable amplification results in almost all PCR applications. The buffer contains a well-balanced ratio of potassium-, ammonium- and magnesium-ions to ensure high specificity and minimal by-product formation without the need of additional optimization steps.
Ruby Buffer additionally contains gel loading buffer and an inherent red dye allowing the direct loading of the PCR product into the gel. The red dye allows an easy visual control during PCR set-up and in combination with the density reagent the direct loading of the reaction product into the gel.
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 at the initial denaturation step.

Content:
Hot Stort Polymerase Ab+ (red cap)
5 units/µl antibody-blocked hot start polymerase in 20 mM Tris-HCl, 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.5 % Tween-20, 0.5 % Nonidet P-40, 50 % (v/v) Glycerol, pH 8.0 (25°C)

dNTP Mix / 10 mM (white cap)
10 mM of dATP, dCTP, dGTP and dTTP

Ruby Buffer (black cap)
10 x conc. complete PCR buffer containing 200 mM Tris-HCl, KCl, $(NH_4)_2SO_4$ and 20 mM $MgCl_2$, red tracking dye and density reagent for gel loading

Crystal Buffer (green cap)
10 x conc. complete PCR buffer containing 200 mM Tris-HCl, KCl, $(NH_4)_2SO_4$ and 20 mM $MgCl_2$

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component	PCR-216S	PCR-216L	PCR-216XL
Hot Start Polymerase Ab+	200 units / 40 µl	1000 units / 200 µl	5000 units / 1 ml
dNTP Mix / 10 mM	200 µl	1 ml	5 x 1 ml
Ruby Buffer	1.2 ml	5 x 1.2 ml	25 ml
Crystal Buffer	1.2 ml	5 x 1.2 ml	25 ml

Assay Set-Up:
Before starting, vortex all components thoroughly to ensure homogeneity.
Prepare a premix for the number of assays you need according to the following protocol:

comp.	cap	stock conc.	final conc.	1 assay @20 µl	1 assay @ 50 µl
PCR-grade Water	white			fill up to 10 µl	fill up to 30 µl
Ruby Buffer or Crystal Buffer	black or green	10x	1x	2 µl	5 µl
dNTP Mix / 10 mM	white	10 mM	200 µM	0.4 µl	1 µl
Hot Start Polymerase Ab+	red	5 units/µl	0.025 units/µl	0.1 µl	0.25 µl
primer mix or each primer		10 µM each primer	200 - 400 nM each primer	0.4-0.8 µl	1 - 2 µl
template /sample DNA				10 µl < 10 ng DNA	20 µl < 20 ng DNA

Select PCR tubes, stipes or plates as recommended for your cycler model.
Aliquot premix into each well and add template DNA (or PCR-grade Water for negative controls).

Cycling Conditions:
Spin down the tubes/plate briefly to remove bubbles and place them into the cycler.

initial denaturation	95 °C	2 min	1x
denaturation annealing[1)] elongation[2)]	95 °C 50 - 68 °C 72 °C	10 - 20 sec 10 - 20 sec 20 sec - 4 min	25 - 35x

[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 amplified. A time of 1 min/kb is recommended.

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

Additional Buffer Systems:
Labeling Buffer (#PCR-263) is recommended for DNA labeling or mutagenesis applications. The buffer is specially optimized for incorporation of labeled or modified nucleotides into DNA. It gives superior results in a broad range of reaction conditions with most primer-template pairs but amplification may also tend to an increased unspecifity.
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:
A final Mg^{2+} concentration of 2.0 mM is recommended in combination with Labeling Buffer. However, if an individual Mg^{2+} optimization is essential add 25 mM $MgCl_2$ stock solution (#PCR-266) as shown in the table below.

final $MgCl_2$ conc.	**20 µl final assay volume**	**50 µl final assay volume**
2 mM	-	-
3 mM	0.8 µl	2.0 µl
4 mM	1.6 µl	4.0 µl
5 mM	2.4 µl	6.0 µl

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Related Products:
Crystal Buffer, #PCR-271
Labeling Buffer, #PCR-263
KCl Buffer, #PCR-262
SYBR DNA Stain, #PCR-273
Gel Loading Buffer with DNA Stain: Blue #PCR-273, Green #PCR-275, Orange #PCR-276
Mg^{2+} Stock, #PCR-266”