DATA SHEET

Jena Bioscience

Hot Start Core Kit Apta+

Kit of aptamer-inhibited hot start DNA polymerase for high specificity, dNTPs and reaction buffer

Cat. No.	Amount
PCR-215S	200 units
PCR-215L	5 x 200 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:
Taq Core Kit Apta+ contains all reagents required for PCR (except template and primer) in one box combining simple handling with high flexibility. Taq Polymerase is recommended for routine PCR applications (up to 4 kb fragment length), high throughput PCR or genotyping. The 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 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 Apta+ requires no prolonged heating or denaturing step. The polymerase inhibiting aptamer is quickly released at the increased temperature of thermal cycling.

Content:
PCR-212
Hot Start Polymerase Apta+ (red cap)
5 units/µl aptamer-inhibited 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)

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 $MgCl_2$

NU-1006
dNTP Mix / 10 mM (white cap)
10 mM of dATP, dCTP, dGTP and dTTP

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component	PCR-215S	PCR-215L
Hot Start Polymerase Apta+	200 units / 40 µl	1000 units / 200 µl
dNTP Mix / 10 mM	400 µl	2x 1 ml
Ruby Buffer	1.2 ml	5 x 1.2 ml
Crystal Buffer	1.2 ml	5 x 1.2 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.	stock conc.	final conc.	1 assay @20 µl	1 assay @ 50 µl
PCR-grade Water			fill up to 10 µl	fill up to 30 µl
Ruby Buffer or Crystal Buffer	10x	1x	2 µl	5 µl
dNTP Mix / 10 mM	10 mM	200 µM	0.4 µl	1 µl
Hot Start Polymerase Apta+	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 $MgCl_2$ concentration of 2.0 mM is recommended in combination with Labeling Buffer. However, if an individual$MgCl_2$ optimization is essential add 25 mM $MgCl_2$ stock solution (#PCR-266) as shown in the table below.

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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

Related Products:
Ruby Buffer #PCR-272
Crystal Buffer #PCR-271
KCl Buffer #PCR-262
SYBR DNA Stain #PCR-273
Gel Loading Buffer with DNA Stain: Blue #PCR-274, Green #PCR-275, Orange #PCR-276
$MgCl_2$ Solution #PCR-266
PCR-grade water #PCR-258
DNA Ladders