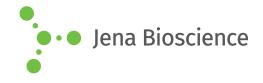
# **DATA SHEET**





## **Hot Start Polymerase**

Heat-activatable DNA polymerase for high specificity, aptamer-inhibited Thermus aquaticus, recombinant, *E. coli* 

Cat. No.	Amount
PCR-212S	200 units
PCR-212L	1.000 units
PCR-212XL	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 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 onset of 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  $5'\rightarrow 3'$  direction in the presence of magnesium. It also possesses a  $5'\rightarrow 3'$  polymerization-dependent exonuclease replacement activity but lacks a  $3'\rightarrow 5'$  exonuclease (proof-reading) activity.

## **Activation step**

Hot Start Polymerase requires no prolonged heating or denaturing step. The polymerase inhibiting aptamer is quickly released at the increased temperature of thermal cycling.

### **Content:**

## Hot Stort Polymerase (red cap)

5 units/ $\mu$ l 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)

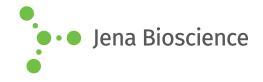
## Ruby Buffer (black cap)

10 x conc. complete PCR buffer containing 200 mM Tris-HCl, KCl, (NH $_4$ ) $_2$ SO $_4$  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<sub>2</sub>

component	PCR-212S	PCR-212L	PCR-212XL
Hot Start Polymerase	200 units / 40 μl	1000 units / 200 μl	5000 units / 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





## **Hot Start Polymerase**

Heat-activatable DNA polymerase for high specificity, aptamer-inhibited Thermus aquaticus, recombinant, *E. coli* 

#### **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:

			<b>.</b> .	_	
comp.	cap	stock	final	1 assay	1 assay
		conc.	conc.	@20 μl	@ 50 μl
PCR-	white			fill up	fill up
grade				to 10 μl	to 30 μl
Water					
Ruby	black	10x	1x	2 μl	5 μl
Buf-	or				
fer or	green				
Crystal					
Buffer					
dNTP	white	10 mM	200 μΜ	0.4 μl	1 μl
Mix /					
10 mM					
#NU-					
1006					
Hot	red	5	0.025	0.1 μl	0.25 μl
Start		units/µl	units/µl		
Poly-					
merase					
primer		10 μM	200 -	0.4-0.8	1 - 2 μl
mix or		each	400 nM	μl	·
each		primer	each		
primer			primer		
template				10 μl <	20 μl <
/sample				10 ng	20 ng
DNA				DNA	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	95 °C	10 - 20 sec	25 - 35x
annealing <sup>1)</sup>	50 - 68 °C	10 - 20 sec	
elongation <sup>2)</sup>	72 °C	20 sec - 4 min	

<sup>&</sup>lt;sup>1)</sup>The annealing temperature depends on the melting temperature of the primers used.

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<sub>2</sub> concentration and annealing temperature.

## Optimization of MgCl<sub>2</sub> concentration:

A final Mg<sup>2+</sup> concentration of 2.0 mM is recommended in combination with Labeling Buffer. However, if an individual Mg<sup>2+</sup> optimization is essential add 25 mM MgCl<sub>2</sub> stock solution (#PCR-266) as shown in the table below.

final MgCl <sub>2</sub> 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 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<sup>2+</sup> Stock, #PCR-266"

<sup>&</sup>lt;sup>2)</sup>The elongation time depends on the length of the fragments to be