



## Taq Polymerase / Labeling Buffer

Thermostable DNA Polymerase  
 Thermus aquaticus, recombinant, *E. coli*

Cat. No.	Amount
PCR-201S	200 units
PCR-201L	1.000 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/ $\mu$ l

### Description:

Taq Polymerase / Labeling Buffer is recommended for DNA labeling or mutagenesis. The buffer system 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 unspecificity.

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.

### Content:

#### Taq Polymerase (red cap)

5 units/ $\mu$ l Taq DNA 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)

#### Labeling Buffer (green cap)

10x conc. complete PCR buffer containing 600 mM Tris-HCl, 150 mM  $(\text{NH}_4)_2\text{SO}_4$ , 20 mM  $\text{MgCl}_2$ , 0.05 % Tween-20, 0.05 % Nonidet P-40, pH 8.8 (25°C)

#### $\text{MgCl}_2$ Stock Solution (yellow cap)

25 mM  $\text{MgCl}_2$

component	PCR-201S	PCR-201L
Taq Polymerase	200 units / 40 $\mu$ l	1000 units / 200 $\mu$ l
Labeling Buffer	1.2 ml	5 x 1.2 ml
$\text{MgCl}_2$ Stock	1.5 ml	4 x 1.5 ml



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### 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
Labeling Buffer	green	10x	1x	2 µl	5 µl
dNTP Mix / 10 mM #NU-1006	white	10 mM	200 µM	0.4 µl	1 µl
dNTP Mix / 10 mM #NU-1006	white	10 mM	200 µM	0.4 µl	1 µl
Taq Polymerase	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, stripes 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>1)</sup>The annealing temperature depends on the melting temperature of the primers used.

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

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

### Additional Buffer Systems:

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.

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  
 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"