



# Taq DNA Pol 5 U/μl with PCR Direct Loading Buffer

Cat. # **11EPTQL025** Pack size **250 U**

### Reference contents:

- > Taq DNA Polymerase 5 U/μl
- > PCR Direct Loading buffer 10xC with MgCl<sub>2</sub>
- > MgCl<sub>2</sub> at 25 mM

Store at **-20°C**.

For research use only.

**Taq DNA Polymerase** is a highly purified thermostable recombinant polymerase, with a 5'-3' exonuclease activity, but no 3'-5' exonuclease activity.

**PCR Direct Loading Buffer 5 x C**, contains incubation buffer for Taq DNA polymerase, densifying agent and red purple dye for direct loading onto agarose gel after PCR cycling. The red purple dye migrates at 400 bp on a 1.2% agarose gel.

### Reaction conditions:

Composition of direct loading buffer diluted to 1x: 10 mM Tris-HCl pH 8.3 (25°C); 50 mM KCl; 1.5 mM MgCl<sub>2</sub>; stabilisers; densifying compound; red-purple loading dye. Taq DNA polymerase is supplied in a formulation containing 50% glycerol. The following information is provided as general advice.

Reagent	20 μl Reaction	50 μl Reaction	Final Conc.
Sterilized water	Add to 20μl	Add to 50μl	
Direct loading buffer 5xC	4 μl	10 μl	1xC
10 mM dNTPs each	0.4 μl	1 μl	200 μM each
Primer A	x μl	x μl	0.2 to 0.5 μM
Primer B	x μl	x μl	0.2 to 0.5 μM
DNA Template *	x μl	x μl	
Taq DNA Pol 5 U/μl	0.2 μl to 0.3 μl	0.2 μl to 0.3 μl	1 to 1.5 U/rxn **

\* Suggested amount of DNA template: 100 pg of plasmid or phage DNA; 10 to 100 ng of genomic DNA; 0.2 to 30 ng of cDNA.

\*\* If PCR inhibitors remain in the reaction mix, higher amounts of Taq DNA Pol may be necessary (2-3 U/rxn) to ensure optimal PCR results. Same advice for amplification of fragments up to 3 – 4 kb.

### Optimisation of Mg concentration:

Most PCR applications are optimal with 1.5 mM MgCl<sub>2</sub>. If a higher MgCl<sub>2</sub> concentration is necessary, use the separate MgCl<sub>2</sub> vial at 25 mM.

Final concentration of MgCl <sub>2</sub> in a 50 μl PCR reaction (mM)	1.5	1.75	2	2.5	3	4
Volume of 25 mM MgCl <sub>2</sub> to add, (μl)	0	0,5	1	2	3	5

### Guidelines for PCR program:

Cycle step	Temperature	Time	Cycles
Initial denaturation(1)	93-95°C	2-4 min	1
Denaturation	93 °C	30 - 60 sec	20 -40
Annealing (2)	(T <sub>m</sub> – 5°C)	30 - 60 sec	
Elongation (3)	72°C	1 min /kb	
Final elongation (4)	72°C	10 min	1
End of PCR assay	+ 4°C / -20°C	Hold / Store	

(1) For GC rich templates, extend to 10 min, but add Taq DNA Pol after initial denaturation or keep maximum 5 min at 95°C by adding up to 10% DMSO to reaction mix.

(2) The annealing temperature depends on the length and the T<sub>m</sub> (melting temperature) of the primers. It should be placed 5°C below the lowest T<sub>m</sub> of the pair of primers. Avoid annealing temperatures under 48°C.

(3) Elongation time will depend on the length of the amplified template. For amplifications less than 1kb, 15 to 30 sec could be enough.

(4) A final elongation step is recommended to fill-in the incompletely amplified fragments and to add the A residues to the 3'ends of PCR products, (useful for T/A cloning protocols).

### Downstream applications:

- > PCR product can directly be re-amplified by "nested PCR"
- > The Direct Loading buffer components do not interfere with restriction enzyme digestions when up to 50 % of the PCR reaction volume is used.
- > Taq DNA polymerase add overhang A on PCR product thus T/A cloning could be performed.
- > If necessary, Red dye can be removed by ethanolic precipitations or by GENE CLEAN turbo PCR cleaning kit.

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### Quality control:

#### Unit assay conditions:

One unit of DNA polymerase is the amount of enzyme required to catalyse the incorporation of 10 nanomoles of nucleosides into a DE81 adsorbable product within 30 min at 74°C under assay conditions.

Absence of contamination: No nickase, endonuclease, 3' exonuclease, ribonuclease activities detected.

PCR assay on human genomic DNA: Specific PCR products of 400 bp using human β-globin gene as a template and decreasing amounts of both DNA template and Taq DNA polymerase are obtained in all assays.

### Troubleshooting:

#### 1. Little or no amplification observed:

- > Increase the elongation time on the basis of 60 sec/kb
- > Check the T<sub>m</sub> of the primers, their quality and their length (> 18 bases for animal material and > 20-22 bases for plants)
- > Avoid primer secondary structures, like dimers, hairpins
- > Avoid any mismatch or point mutations in the 3' region of the primers
- > Avoid 3 G or 3C residues in the 3'end region of the primer
- > Lower the annealing temperature by 2°C to 5°C steps
- > If annealing temperature (T<sub>a</sub>) is close to the elongation temperature, perform PCR by decreasing "T<sub>a</sub>" every cycle
- > Increase the number of cycles by 5 (if < 35)
- > If templates are GC rich or contain difficult secondary structures, add DMSO up to 10% by 2% steps
- > Increase the quantity of the template, and/or the primers
- > Consider the presence of inhibitory factors

#### 2. Multiple bands or smearing observed:

- > Check the T<sub>m</sub> of the primers, their design, their length, their quality
- > Check the homologous sequences of the primers to the template
- > Verify the absence of secondary structures, like hairpins
- > Increase the annealing temperature by 2°C to 5°C steps
- > Lengthen the homologous sequence of the primer to the template
- > Reduce the number of cycles by 5
- > Reduce the concentration of DNA template and/or primers