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

Cat. # **Pack size**
11EPTQL100 **1000 U**

Reference contents:

- *Taq* DNA Polymerase 5 U/µl
- PCR Direct Loading buffer 10xC with $MgCl_2$
- $MgCl_2$ 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 1xC: 10 mM Tris-HCl pH 8.3 (25°C); 50 mM KCl; 1.5 mM $MgCl_2$; 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 $MgCl_2$. If a higher $MgCl_2$ concentration is necessary, use the separate $MgCl_2$ vial at 25 mM.

Final concentration of $MgCl_2$ in a 50 µl PCR reaction (mM)	1.5	1.75	2	2.5	3	4
Volume of 25 mM $MgCl_2$ 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 Annealing (2) Elongation (3)	93 °C (Tm – 5°C) 72°C	30 - 60 sec 30 - 60 sec 1 min /kb	20 -40
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 Tm (melting temperature) of the primers. It should be placed 5°C below the lowest Tm 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 GENECLEAN 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 Tm 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 (Ta) is close to the elongation temperature, perform PCR by decreasing "Ta" 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 Tm 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