Taq Core Kits 25

Cat. # 11EPTQK251

Contents	
No.of Tubes	**Components**
1	250U *Taq* DNA Polymerase at 5 U/µl
1	dNTPs mix at 25mM each
2	PCR buffer 10xC with $MgCl_2$
1	PCR buffer 10xC without $MgCl_2$
1	$MgCl_2$ at 25 mM

Store at –20°C.

For research use only.

***Taq* Core Kits 25** contain all reagents required for PCR but as separated items:

- *Taq* DNA Polymerase, a highly purified thermostable recombinant polymerase, with a 5'-3' exonuclease activity, but no 3'-5' exonuclease activity. *Taq* DNA Polymerase resists to prolonged incubations at 95°C.
- Highly purified dNTPs in a ready-to-use mastermix at 25 mM each, guaranteed for PCR.
- Optimised standard PCR buffers 10xC provided with and without $MgCl_2$, highly efficient in most PCR conditions.
- $MgCl_2$ solution at 25mM as a separate item for peculiar optimisation of $MgCl_2$ concentration.

Reaction conditions:

Composition of PCR buffer with $MgCl_2$ diluted to 1xC: 10 mM Tris-HCl pH 9.0 (25°C); 50 mM KCl; 1.5 mM $MgCl_2$; 0.1% Triton X100; 0.2 mg/ml BSA.

Taq DNA polymerase is supplied in a formulation containing 50% glycerol.
Composition of storage buffer: 20mM Tris-HCl pH 8.0; 100mM KCl; 0.1mM EDTA; 1mM Dithiothreitol; 0.5% Tween 20; 0.5% Nonidet P40; 50% glycerol.

The following information is provided as general advice. When setting up multiple reactions, the preparation of a reaction mastermix containing all non variable components is recommended. That will reduce risks of cross contamination and errors when pipetting low volumes.

Reagent	20 µl Reaction	50 µl Reaction	Final Concentration
Sterilized water	Add to 20µl	Add to 50µl	
PCR buffer 10xC	2 µl	5 µl	1xC
dNTPs mix 25 mM each	0.16 µl	0.4 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.
***Ideally, prepare a 10x solution containing both PCR primers.

Optimisation of Mg concentration:

Most PCR applications are optimal with 1.5mM $MgCl_2$. If optimisation of $MgCl_2$ concentration is necessary, use the PCR buffer without $MgCl_2$ and the separate $MgCl_2$ vial at 25mM.
The recommended range of $MgCl_2$ concentration is between 1mM and 4mM.

Final concentration of $MgCl_2$ (mM)	1	1.25	1.5	1.75	2	2.5	3	4
Volume (µl) of 25mM $MgCl_2$ in a 20 µl PCR reaction	0.16	1	1.2	1.4	1.6	2	2.4	3.2
Volume (µl) of 25mM $MgCl_2$ in a 50 µl PCR reaction	2	2.5	3	3.5	4	5	6	8

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).

Characteristics of dNTPs:

- dNTPs Mix 25mM is a premixed solution in a TE Buffer (Tris-HCl 10mM, EDTA 1mM, pH7.5), containing the sodium salts of 4 nucleotides (dATP, dCTP, dGTP, dTTP), each at a concentration of 25mM.
- The individual dNTPs, PCR grade, are guaranteed of high purity as measured by HPLC (dATP, dCTP, dGTP, and dTTP HPLC areas % >99%).
- The solution is specially manufactured and tested for application in PCR, RT-PCR, qPCR, DNA Labelling and sequencing.

Guidelines for primer sequence design:

- Tm (melting temperature) of both primers should be equivalent and not exceed 70°C.
- Annealing temperature (Ta) should be about 5°C below the lowest Tm of the pair of primers.
- One primer length will depend upon its base content and the Tm of its partner.
- The likelihood of annealing to sequences other than the chosen target has to be very low. The size of the primers must be over 16-18 bases for human or animal templates and over 20-22 bases for plants. The length should not exceed 28-30 bases.
- Final base composition should always be 50-60% (G+C).
- 3' ends of primers should not be complementary to avoid primer dimers.
- Avoid 3 G or C residues in the 3' end region of the primer, as it may result in non specific annealing.
- Ability to form secondary structures such as hairpins should be avoided.

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 absorbable 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: *Taq* DNA Polymerase, PCR buffers and dNTPs mix, are analysed by PCR to check batch to batch uniformity. Specific PCR products of 400 bp using a human ß-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.
- Increase the amount of *Taq* DNA Polymerase directly up to 2U/rxn or in 0.5 U steps.
- Increase the concentration of $MgCl_2$ in 0.25mM steps (see the table for optimisation of Mg concentration).
- Check the Tm of the primers, their quality and their length (>18 bases for animal material and >20-22 bases for plants).
- Avoid secondary structure primers, like dimers, hairpins.
- Avoid any mismatch or point mutations in the 3' region of the primers.
- Avoid 3G or 3C residues in the 3'end region of the primer.
- Both primers might be present at the same final concentration.
- Prevent primers degradation by making aliquots and storing them at -20°C.
- 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).
- Make sure that the final elongation of 10 min at 72°C has been programmed.
- If templates are GC rich or contain difficult secondary structures, add DMSO up to 10% by 2% steps. Reduce enzyme concentration (0.5 U/rxn).
- Increase the quantity of the template, and/or the primers.
- Verify the quality of your DNA template and check for possible degradation onto an agarose gel.
- When using cDNA as a template, check the quality of your RNA and consider the performance of RT step. Load an aliquot of cDNA onto an agarose gel.
- The volume of RT-reaction (cDNA) should not exceed 10% of the PCR reaction volume.
- Consider the presence of inhibitory factors.
- Use thin-wall PCR tubes.

2. Multiple bands or smearing observed:
- Decrease the amount of *Taq* DNA Polymerase in 0.5 U steps.
- Check the Tm of the primers, their length and their quality.
- Check the homologous sequences of the primers to the template.
- Verify the absence of secondary structures, like hairpins.
- Both primers might be present at the same final concentration.
- 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.
- Use serial dilution of DNA template.

Other PCR buffers available from MP Biomedicals:

- **PCR buffer XD** formulated without BSA and without detergents, to work on plant or animal material or using further dHPLC.
- **$(NH_4)_2SO_4$ PCR buffer** containing ammonium sulphate to improve efficiency and specificity of some applications.
- **Direct loading buffer** with a densifying agent and a red purple dye that allows direct monitoring on agarose gels.

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