



## Taq Core Kit 25 Direct Loading

Cat. # **11EPTQKL251**      Pack size **250 U**

### Kit contents:

| Contents     |   |
|--------------|---|
| No. of Tubes | Components  |
| 1            | 250U <i>Taq</i> DNA Polymerase at 5 U/μl                |
| 1            | dNTPs mix at 25mM each                                  |
| 2            | PCR Direct loading buffer 5xC with MgCl <sub>2</sub>    |
| 2            | PCR Direct loading buffer 5xC without MgCl <sub>2</sub> |
| 1            | MgCl <sub>2</sub> at 25 mM                              |

Store at **-20°C**.

For research use only.

**Taq Core Kits 10** 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 10 mM or 25 mM each, guaranteed for PCR.
- Optimised PCR Direct Loading buffers 5xC provided with and without MgCl<sub>2</sub>, highly efficient in most PCR conditions.
- MgCl<sub>2</sub> solution at 25 mM as a separate item for peculiar optimisation of MgCl<sub>2</sub> concentration

**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<sub>2</sub>; stabilisers; densifying compound; red-purple loading dye.

*Taq* DNA polymerase is supplied in a formulation containing 50% glycerol.

Composition of storage buffer: 20 mM Tris-HCl pH 8.0; 100 mM KCl; 0.1 mM EDTA; 1 mM 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      |                     |
| Direct Loading buffer 5xC | 4 μl             | 10 μl            | 1xC                 |
| dNTPs mix 25 mM 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             |                     |
| <i>Taq</i> 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 1.5 mM MgCl<sub>2</sub>. If optimisation of MgCl<sub>2</sub> concentration is necessary, use the PCR buffer without MgCl<sub>2</sub> and the separate MgCl<sub>2</sub> vial at 25 mM. The recommended range of MgCl<sub>2</sub> concentration is between 1 and 4 mM.

|  |      |      |     |      |     |     |     |     |
|--|------|------|-----|------|-----|-----|-----|-----|
| Final concentration of MgCl <sub>2</sub> (mM)                  | 1    | 1.25 | 1.5 | 1.75 | 2   | 2.5 | 3   | 4   |
| Volume (μl) of 25 mM MgCl <sub>2</sub> in a 20 μl PCR reaction | 0.16 | 1    | 1.2 | 1.4  | 1.6 | 2   | 2.4 | 3.2 |
| Volume (μl) of 25 mM MgCl <sub>2</sub> 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             | 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 GENECLEAN turbo PCR cleaning kit.

## **Characteristics of dNTPs:**

- dNTPs Mix 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 either 10 mM or 25 mM
- 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 Labeling and sequencing.

## **Guidelines for primer sequence design:**

- T<sub>m</sub> (melting temperature) of both primers should be equivalent and not exceed 70°C
- Annealing temperature (T<sub>a</sub>) should be about 5°C below the lowest T<sub>m</sub> of the pair of primers.
- One primer length will depend upon its base content and the T<sub>m</sub> 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 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: *Taq* DNA Polymerase, Direct Loading buffers and dNTPs mix, are analysed by PCR to check batch to batch equivalence. Specific PCR products of 400 bp using a human  $\beta$ -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<sub>2</sub> in 0.25 mM steps (see the table for optimisation of Mg concentration)
- 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
- 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 (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)
- 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 by loading 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 T<sub>m</sub> of the primers, their length, 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**

- **Standard PCR buffer**, with or without MgCl<sub>2</sub>, used in a broad range of PCR conditions.
- **PCR buffer XD** formulated without BSA and without detergents, to work on vegetal or beef material or using further dHPLC
- **(NH<sub>2</sub>)SO<sub>4</sub> PCR buffer** containing ammonium sulphate to improve efficiency and specificity of some applications

For further Information contact us:

### **MP Biomedicals Europe**

Tel: 00800.7777.9999

Fax: 00800.6666.8888

[custserv.eur@mpbio.com](mailto:custserv.eur@mpbio.com)

### **MP Biomedicals North America**

Tel: 800 854 05 30

Fax: 800 934 69 99

[custserv@mpbio.com](mailto:custserv@mpbio.com)

[www.mpbio.com](http://www.mpbio.com)