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# ON Taq Master Mix

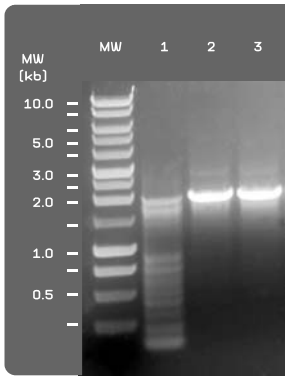
## Automatic "Hot Start" PCR system

### onTaq Master Mix (2x)

*(Thermus aquaticus)*

| Cat. No. | Size |
|---|---|
| E2714-01 | 100 reactions |
| E2714-02 | 200 reactions |
| E2714-03 | 500 reactions |

**Storage Conditions:** Store at -20˚C for long term storage, or at +4˚C for up to two months.




**PCR amplification using ON Taq Master Mix.** A 2 kb amplicon of the human beta-globin gene was amplified using onTaq PCR Master Mix (2x).
Lane M: molecular size marker- Perfect Plus 1 kb DNA Ladder (E3131).
Lane 1: PCR amplification reaction using Taq PCR Master Mix (2x). Reaction was incubated 30 min at 25˚C before PCR.
Lanes 2, 3: PCR amplification reactions using ON Taq PCR Master Mix (2x). Reactions were incubated 30 min at 25˚C before PCR.

**Enzyme Properties:**

| Property | Value |
|---|---|
| Template | ssDNA, dsDNA |
| 5'-3' exonuclease | yes |
| 3'-5' exonuclease | no |
| Proofreading | no |
| Strand displacement | no |
| Error rate | $>10^{-6}$ |
| Relative Accuracy Ratio* (*Taq* = 1) | 1 |
| Half life at 95˚C | 40 min |
| Amplicon length | up to 10 kb |
| Generation of 3'-A-overhangs | yes (to a fraction of amplicons) |
| TA- / blunt cloning possible | yes / yes |

*Relative accuracy ratio := Error rates onTaq / *Taq*. A value of 10 indicates 10-fold higher accuracy as compared to *Taq* DNA Polymerase, a value of 1 indicates similar accuracy / precision.

**Top quality, recombinant thermostable *Taq* DNA polymerase for automatic "hot start" PCR. Non-antibody mediated, chemically inhibited "HotStart".**

**Description:**

- onTaq PCR Master Mix (2x) is a ready-to-use solution containing onTaq DNA Polymerase, optimized reaction buffer, $MgCl_2$ and dNTPs.
- Use of onTaq PCR Master Mix (2x) saves time, increases reproducibility (due to avoiding calculation and pipetting errors) and reduces contamination risk (due to fewer pipetting steps) during PCR set-up.
- onTaq DNA Polymerase is a modified „hot start" Taq DNA polymerase. The enzyme provides very tight inhibition of the polymerase activity at moderate temperatures and allows room temperature reaction setup.
- Polymerase activity is restored during the initial denaturation step when amplification reactions are heated at 95˚C for 10 minutes.
- "Hot start" PCR may increase specificity, sensitivity and yield of a PCR reaction in comparison to the conventional PCR assembly method.
- Thermostable onTaq DNA Polymerase replicates DNA at 72˚C and exhibits a half-life of 40 min at 95˚C.
- Catalyzes the polymerization of nucleotides into duplex DNA in 5' → 3' direction in the presence of magnesium ions.
- Contains 5' → 3' exonuclease activity.
- Lacks 3' → 5' exonuclease activity ("proofreading").
- Capable of adding extra A to a fraction of 3' ends.
- onTaq DNA Polymerase is recommended for use in PCR and primer extension reactions at elevated temperatures to obtain a wide range of DNA products up to 10 kb in length.

**onTaq PCR Master Mix (2x) contains:**

1. onTaq PCR Master Mix (2x)
2. Water, nuclease free
3. 10 x Color Load

**onTaq PCR Master Mix (2x):**

onTaq DNA Polymerase is supplied in 2 x Pol Buffer B containing 3 mM $MgCl_2$ and 0.4 mM of each dNTP.
Final concentrations: 1.5 mM $MgCl_2$ and 0.2 mM of each dNTP.

**10 x Color Load:**

10 x Color Load contains two gel tracking dyes and a gel loading reagent.
It enables direct loading of PCR products onto agarose gels.

**Quality Control:**

All preparations are assayed for contaminating endonuclease, 3'-exonuclease, and nonspecific single- and double-stranded DNase activities. Typical preparations are greater than 95 % pure, as judged by SDS polyacrylamide gel electrophoresis.

**References:**


1. *Chien, A., Edgar, D.B. and Trela, J.M. (1976) J. Bacteriol. 127, 1550.*
2. *Kaledin, A.S., Sliusarenko, A.G. and Gorodetskii, S.I.(1980) Biokhimiya 45, 644.*



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## "HOT START" PCR PROTOCOL

**Preparation of PCR Reaction:**

| Component | Volume/reaction | Final concentration |
|---|---|---|
| ON Taq PCR Master Mix (2x) | 25 µl | 1.25 U onTaq DNA Polymerase<br>1 x Reaction Buffer (1.5 mM $MgCl_2$)<br>0.2 mM of each dNTP |
| Upstream primer | Variable | 0.1-0.5 µM |
| Downstream primer | Variable | 0.1-0.5 µM |
| Optional:<br>10 x Color Load | 5 µl | 1 x |
| Template DNA | Variable | <0.5 µg/50 µl |
| Sterile double-distilled water | To 50 µl | - |
| Total volume | 50 µl | - |

General formula for calculating total gene / genome copy numbers from the total amount of template DNA::

$$Template\ DNA\ copy\ number\ [molecules] =$$

$$\frac{DNA\ amount\ [ng] \cdot\ 6.022x10^{23}\ [molecules\ mol^{-1}]}{Genomic\ DNA\ length\ [kb] \cdot\ 616\ [g\ mol^{-1}\ bp^{-1}]} \cdot \frac{10^{-3}\ [kb\ bp^{-1}]}{10^{9}\ [ng\ g^{-1}]}$$

Optimum: $10^4$ template DNA copies
Maximum: 0.5 µg template DNA or less

**Thermal Cycling Conditions:**

| Step | Temperature | Time | Number of Cycles |
|---|---|---|---|
| Initial Denaturation | 95°C | 10 min | 1 |
| Denaturation | 94°C | 30 s | 25-35 |
| Annealing | 50-68°C | 30 s | |
| Extension | 72°C | 1 min/1 kb | |
| Final Extension | 72°C | 7 min | 1 |
| Cooling | 4°C | Indefinite | 1 |

**Notes:**

1. **First Law of PCR:** PCR is comparable to homeopathic processes, working best, if *all* components are added in homeopathic dosages only. Any PCR reaction component, if introduced in excess amounts, beyond the borders of specification, may impair or inhibit the PCR reaction.
2. **Concentration Differences:** Completely thaw and mix thoroughly all components of PCR reaction before use to avoid localized differences in salt concentration. It is especially important for magnesium solutions, because they form concentration gradient when frozen.
3. **Assembly at Room Temperature**: Prepare reaction mixes at room temperature. Use of onTaq DNA Polymerase allows room temperature reaction setup. Mix well.
4. **Primer Mix.** Primers can be added separately or as a previously prepared primer mix.
5. **Mix Template:** Vortex the samples and briefly spin down.
6. **Cycler Preheating Not Required:** Reactions are placed in a non preheated (room temperature) thermal cycler.
7. **$MgCl_2$:** Standard concentration of $MgCl_2$ in PCR reaction is 1.5 mM (as provided in 1x Pol buffers B and C) when using 0.2 mM dNTP (each). In most cases this concentration will produce satisfactory results. However, in some cases, reaction may be improved by determining the optimal concentration of $MgCl_2$. dNTP concentrations exceeding 0.2 mM (each) would require additional $MgCl_2$.
   Adding 1 µl of a 25 mM $MgCl_2$ solution to a total reaction volume of 50 µl will add 25 nmol $MgCl_2$ and thus increase total $Mg^{2+}$-reaction concentration in 0.5 mM.
   Increasing the $Mg^{2+}$ concentration enhances PCR yield but decreases reaction specificity (amplification of more bands, but also of non-specific bands). Decreasing $Mg^{2+}$-concentration decreases PCR yield but enhances reaction specificity (less bands, but specific PCR products).
8. **Color Load.** Use of the 10 x Color Load allows PCR reactions to be loaded directly onto an agarose gel without prior addition of a gel loading buffer.The 10 x Color Load contains a gel loading reagent and two gel tracking dyes (a red dye and an yellow dye) that separate during electrophoresis. In a 1 % agarose gel, the red dye migrates at the same rate as 600 bp DNA fragment and the yellow dye migrates faster than 20 bp. The dyes do not interfere with most downstream enzymatic applications, however it is recommended to purify PCR products prior to enzymatic manipulation.
9. **PCR additives / PCR Enhancers.** Most often, addition of additives / PCR enhancers is not required. For some difficult targets such as: GC-rich sequences, sequences with complex secondary structures, additives such as DMSO can be included to improve amplification. Use DMSO in concentrations of 2-8% [v/v]. The recommended starting DMSO concentration (if required) is 3% [v/v].
10. **Template Copies:** As a general guide for how much template DNA to use, start with a minimum $10^4$ copies of the target sequence to obtain a signal in 25-35 cycles (i.e. 1 µg of 1 kb ds DNA equals 9.8 x $10^{11}$ molecules, 1 µg of *E. coli* genomic DNA equals 2 x $10^8$ molecules, 1 µg of human genomic DNA equals 3 x $10^5$ molecules). Increased amounts of template DNA (> 0.5 µg) negatively affects / inhibits PCR reactions.

**Notes:**

1. **Initial Denaturation.** A 10 min initial denaturation step at 95°C is required to activate ON Taq DNA Polymerase.
2. **Annealing.** Annealing temperature should be optimized for each primer set based on the primer $T_m$. Optimal annealing temperatures may be above or below the estimated $T_m$. As a starting point, use an annealing temperature 5°C below $T_m$.
3. **Long PCR.** When amplifying long PCR products ( > 5 kb):, use an elongation temperature of 68°C instead of 72°C.

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