# TruTaq Green PCR Master Mix

FB02002020 200 Reactions

Store at -25°C to -15°C

TruTaq Green PCR Master Mix is a ready-to-use solution containing TruTaq DNA polymerase, optimized TruTaq Green buffer, $MgCl_2$, and dNTPs. It includes a density reagent and two tracking dyes for monitoring electrophoresis progress: The blue dye migrates with 3-5 kb DNA fragments in a 1% agarose gel and the yellow dye migrates faster than 10 bp DNA fragments in 1% agarose gel. The dyes have absorption peaks at 424 nm and 615 nm.

TruTaq Green PCR Master Mix retains all the features of TruTaq DNA Polymerase. It is compatible with downstream workflows such as DNA sequencing, ligation and restriction digestion. Suitable applications include:

- Routine PCR amplification of DNA fragments up to 6 kb from genomic DNA and up to 20 kb from viral DNA.
- RT-PCR.
- Genotyping.
- Generation of PCR products for TA cloning.

## Kit Contents

| Reagents | 200 Reactions | Description |
|---|---|---|
| 2X TruTaq Green PCR Master Mix | 4 x 1.25 mL | Contains dATP, dCTP, dGTP and dTTP, 0.4 mM each, and 4 mM $MgCl_2$. |
| Water, nuclease-free | 4 x 1.25 mL | - |

## PCR Reaction Setup:

For multiple reactions, prepare a master mix of components common to all reactions to minimize pipetting error, then dispense appropriate volumes into individual PCR tube before adding template DNA.

| Component | 50 µL Reaction | Final Concentration |
|---|---|---|
| Water, nuclease-free | to 50 µL | - |
| 2X TruTaq Green PCR Master Mix | 25 µL | 1X |
| Forward Primer | varies | 0.1-1.0 µM* |
| Reverse Primer | varies | 0.1-1.0 µM* |
| Template DNA | varies | 0.1-1 µg (gDNA) or 0.01-1 ng (plasmid DNA) |

* For degenerate primers and primers used for long PCR, higher primer concentrations in the range of 0.3-1 µM is recommended.

## Thermal Cycling Conditions on Thermal Cycler:

| Step | | Temp. | Time | Recommendation |
|---|---|---|---|---|
| Initial denaturation | | 95°C | 1-3 min | Extend to 10 min for GC-rich templates |
| 25-40 PCR cycles | Denature | 95°C | 30 sec | Extend to 3-4 min for GC-rich templates |
| | Anneal | Tm-5°C | 30 sec | Optimize if non-specific products appear |
| | Extend | 72°C | 1 min | Extend 1 minute/kb for longer product >2 kb<br>Reduce temperature to 68°C for templates >6 kb |
| Final extension | | 72°C | 5-15 min | Extend to 30 minutes for TA cloning to ensure complete 3'-dA tailing of the PCR product |

Load 5-15 µL of PCR mixture directly on a gel.