# Instructions for use

## 2x qPCR CybrGreen Apta MasterMix

**1 ml, 100 reactions**

**Optimized 2x PCR MasterMix for all qPCR assays including aptamer-blocked HotStart Taq DNA polymerase**

### 1. Description

Our ***2x qPCR CybrGreen Apta Master Mix*** is an optimized ready-to-use mixture for all qPCR applications. It contains a modified fast aptamer-blocked HotStart Taq DNA Polymerase, dNTPs and $MgCl_2$ and the fluorescent dye SYBR® Green combined in an optimized buffer system for Real-Time PCR / qPCR applications. You only need to add primers and template DNA. The Master Mix can be used not only for expression analysis but also for genotyping, copy-number analyses and all sorts of quantitative PCRs.

For research use only. Not approved for use in clinical or *in vitro* diagnostics.

### 2. Applications

***2x qPCR CybrGreen Apta MasterMix*** is recommended for use in all qPCR applications. The optimized buffer system provides fast kinetics and highly efficient target amplification with low DNA template amounts and even for difficult templates. The ***2x qPCR CybrGreen Apta MasterMix*** contains all components, you just need to add primers and template DNA / cDNA. The ***2x qPCR CybrGreen Apta MasterMix*** is not only suitable for expression analysis, but also for genotyping, copy-number analysis and all sorts of quantitative PCR assays. Using our ***2x qPCR CybrGreen Apta MasterMix*** not only reduces contamination risks, but is also time saving, highly reproducible and very easy to prepare.

### 3. Set contents

***2x qPCR CybrGreen Apta MasterMix*** in **2x reaction buffer** containing the modified HotStart Taq polymerase, 0.4 mM each dNTP and an optimized buffer system containing $MgCl_2$ and SYBR® Green.

| Reagent | Amount | Lid colour |
|---|---|---|
| ***2x qPCR CybrGreen Apta MasterMix*** (100 reactions) | 1 tube, 1 ml | brown |

**Note:** Some qPCR cyclers require the addition of ROX. The ***2x qPCR CybrGreen Apta MasterMix*** is also available with low or high concentrations of ROX.

### 4. Protocol

**Before you start:** Thaw the tube and invert the MasterMix 5-6 times to ensure mixing of the solution. Do not vortex! After thawing spin the tube briefly!

**Note:** Reactions can be conveniently set up at room temperature.

Recommended reaction mixture per well:

| Components | Apply for 20 µl reaction | Apply for 10 µl reaction | Final concentration |
|---|---|---|---|
| ***2x qPCR CybrGreen Apta MM*** | 10 µl | 5 µl | 1x |
| Forward primer | variable (e.g. 2 µl) | variable (e.g. 1 µl) | 0.1 – 0.4 µM |
| Reverse primer | variable (e.g. 2 µl) | variable (e.g. 1 µl) | 0.1 – 0.4 µM |
| Template DNA | variable | variable | 1 pg – 10 ng/reaction |
| **Sterile dest. water** | **adjust to 20 µl** | **adjust to 10 µl** | |



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- **3-Step PCR protocol**

| Step | Time | Temperature |
|---|---|---|
| Initial denaturation | 2 minutes | 92-95 °C |
| **30 - 45 cycles** | | |
| Denaturation | 5 seconds | 92-95 °C |
| Annealing | 5 seconds | 60 °C (depends on primers) |
| Extension | 5 – 20 seconds | 72 °C |

- **2-Step PCR protocol**

| Step | Time | Temperature |
|---|---|---|
| Initial denaturation | 2 minutes | 92 - 95 °C |
| **30 – 45 cycles** | | |
| Denaturation | 5 seconds | 92 - 95 °C |
| Annealing/Extension combined | 5- 30 seconds | 60 °C (depends on primers) |

**Note:** For maximum efficiency and specificity, annealing temperatures, as well as extension time, primer concentration and template DNA concentration may need to be optimized.

### 5. Storage conditions

Long-term storage: at -20°C (stable for about 24 months).
Short-term storage: at 4°C (stable for about 3 months).
However, short term storage for a few hours or up to 3 days at room temperature will not affect the performance of the MasterMix.





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