Real-time-PCR Master Mix E3  
Cat. No: S170  100 rcs (2x1,25 ml)

**Features:**
- The Master mix contains dUTP instead of dTTP
- The Mix contains ROX (500nM) as passive Reference dye (it provides a baseline in multiplex reactions)
- It contains EvaGreen as fluorescent dye
- The qPCR / RTD-PCR Master mix E3 is ready-to-use and is optimized for high specificity and sensitivity because of optimized reaction buffer
- easy to use because ready-to-use Master Mix

**Applications:**
- Detection and quantification of DNA and cDNA targets
- Profiling gene expression
- Microbial detection
- Viral load determination

**Description:**
The Master Mix contains all reagents required for qPCR (except template and primer) in a premixed 2x concentrated ready-to-use solution. The high specificity and sensitivity of the mix is achieved by an optimized hot-start polymerase. Its activity is blocked at ambient temperature and switched on automatically at the onset of the initial denaturation. The thermal activation prevents the extension of non-specifically annealed primers and primer-dimer formations at low temperatures during PCR setup.
The mix offer dUTP instead of dTTP to prevent carry-over contaminations of DNA from previous PCR reactions.

**Concentration:** The Mastermix is 2x concentrated

**List of components qPCR / RTD-PCR Master mix:**
Hot-Start Polymerase for qPCR, dATP, dCTP, dGTP, dUTP, EvaGreen, ROX, optimized reaction buffer with KCl and MgCl2, stabilizers and enhancers, PCR-grade water

**Transportation:** with blue ice

**Storage:** at 4°C for 3 months, at -20°C for more than 12 months, **Note:** protect from Light

**Usage:**

<table>
<thead>
<tr>
<th>Components</th>
<th>Volume per reaction</th>
<th>final conc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2X qPCR / RTD-PCR Master mix E3</td>
<td>25 µl</td>
<td>1x</td>
</tr>
<tr>
<td>Up-stream primer (10 µM stock)</td>
<td>1.5 µl (range: 0.5-2.5 µl)</td>
<td>300 nM</td>
</tr>
<tr>
<td>Down-stream primer (10µM stock)</td>
<td>1.5 µl (range: 0.5-2.5 µl)</td>
<td>300 nM</td>
</tr>
<tr>
<td>Template DNA</td>
<td>5 µl (0.1-15 ng/ml plasmid DNA) (1-10 µg/ml genomic DNA)</td>
<td>&lt; 500ng DNA</td>
</tr>
<tr>
<td>Sterile dest. Water (included)</td>
<td>up to 50 µl total reaction volume</td>
<td></td>
</tr>
</tbody>
</table>

- vortex all solutions carefully before using and before PCR
- may you add the enzyme mix after Template DNA
- an individual optimization of annealing temperature may be necessary for new combinations of primers and Template DNA
General Thermo-Cycler protocol:

**Note:** working with EvaGreen just select the optical setting for FAM or SYBR Green at the cycler

<table>
<thead>
<tr>
<th>Step</th>
<th>Time</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>UNG treatment (optional)</td>
<td>1x2 min</td>
<td>50°C</td>
</tr>
<tr>
<td>Initial denaturation</td>
<td>1-3 min</td>
<td>95°C</td>
</tr>
</tbody>
</table>

**30-40 Cycles:**
- Denaturation 15-30 sec
- Annealing 30-65 sec
- Extension 30 sec (per 500bp)

**Note:**
- an individual optimization of annealing temperature may be necessary for new combinations of primers and Template DNA

Ordering information.

<table>
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<tr>
<th>Cat.-no</th>
<th>Description</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>S170</td>
<td>Real time PCR Mastermix E3</td>
<td>100 rcs / 50µl</td>
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</table>