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# BioMaster RT-PCR – Standard (2×)

Cat. Number: RM03-80, RM03-400

**Product description:**

**BioMaster RT-qPCR (2×)** reagent kit includes **2× buffer for RT-qPCR** containing all components (except for RNA template and primers), **25× BioMaster-mix** and **DEPC-treated water**. The kit is designed for one-step reverse transcription and real-time polymerase chain reaction (RT-qPCR) with fluorescent probes.

**BioMaster-mix** contains the optimal ratio of M-MuLV –RH to *HS-Taq* DNA polymerase for both reactions.

M-MuLV –RH is a genetically modified Moloney Murine Leukemia Virus reverse transcriptase (M-MuLV). The enzyme exerts RNA- and DNA-dependent polymerase activity but lacks RNase H activity. M-MuLV –RH reverse transcriptase exhibits improved thermal stability and is active at high temperatures (up to 50°C).

*HS-Taq* DNA polymerase is a recombinant *Taq* DNA polymerase inactivated by specific monoclonal antibodies. The enzyme is inactive at temperatures up to 70 °C, it is activated at the first PCR cycle during a short 5-min incubation at 95 °C. Recombinant *HS-Taq* DNA polymerase catalyzes 5′→3′ synthesis of DNA and possesses 5′→3′ exonuclease activity of the native *Taq* DNA polymerase from *Thermus aquaticus*. The recombinant *HS-Taq* DNA polymerase is ideal for standard PCR from matrix up to 5 kbp.

**2× buffer for RT-qPCR** is optimized for both efficient RT and PCR.

**Product composition:**

| Cat. # | 2× buffer for RT-qPCR | 25× BioMaster-mix | DMSO | DEPC-treated water | Number of reactions (25 µl each) |
|---|---|---|---|---|---|
| RM03-80 | 2 × 0.5 ml | 1 × 80 µl | 0.2 ml | 2 × 0.5 ml | 80 |
| RM03-400 | 4 × 1.25 ml | 1 × 400 µl | 0.5 ml | 3 × 1.8 ml | 400 |

**RT-qPCR buffer (2×) contains:**

100 mM Tris-HCl (pH 8.3 at 25 °C), 150 mM KCl, 0.6 mM each deoxynucleoside triphosphate, 0.6 mM $MgCl_2$, 8 mM DTT, enzyme stabilizers and enhancers.

**BioMaster-mix contains:**

50 mM Tris-HCl (pH 8.0 at 25 °C), 100 mM NaCl, 1 mM EDTA, 5 mM DTT, 50 % (v/v) glycerol and 0.1 % (v/v) NP-40, M-MuLV –RH reverse transcriptase, highly-processive HS-*Taq* DNA polymerase and inhibitor of RNases.

**Applications:**

- Gene expression analysis;
- One-step RT-qPCR.

**Reaction mix features**

- The reaction mix is optimized for the specific and effective performance of M-MuLV –RH reverse transcriptase and *HS-Taq* DNA polymerase;
- The mix contains substances that allow long-term storage (storage of **BioMaster RT-qPCR (2×)** at room temperature for 2 days does not affect RT-PCR efficacy) and multiple thawing-freezing cycles;
- Does not contain dyes, which makes the reaction mix multi-purpose.

**Benefits of use**

- High specificity;
- High sensitivity;
- Convenient and easy-to-use;
- Low pipetting error and low risk of cross-contamination;
- Standardized conditions of the same-type reactions (reduced pipetting error during mixing PCR components in a series of experiments);
- PCR products can be further subjected to TA cloning due to deoxyadenosine overhangs at the ends of amplified DNA fragments.

**Limits of use**

- Not recommended to use for amplicons of > 5 kbp.

**Protocol**

Before starting to work, we recommend to get acquainted with the protocol at our site: http://biolabmix.ru/catalog

1. Thaw **2× buffer for RT-qPCR** and vortex thoroughly.
2. Add the following components into the thin-wall PCR tubes considering the final volume of the reaction mixture equal to 25 µl:

| Component | Volume | Final concentration |
|---|---|---|
| 2× mix for RT-qPCR | 12,5 µl | 1× |
| BioMaster-mix | 1 µl | |
| Forward primer | variable | 0.1 – 600 nM |
| Reverse primer | variable | 0.1 – 600 nM |
| Probe | variable | 0.1 – 300 nM |
| RNA template | variable | 1 pg – 1 µg |
| Sterile water | up to 25 µl | |

**Note:** in case of amplification of matrices with complex spatial structure, it is allowed to add DMSO from 1 to 5% of the final volume of the reaction mixture. In this case, take into account the change in Tm of primers when designing the programme.

**Note:** depending on the copy number and complexity of the gene, the added volume of **BioMaster Mix** may vary from 0.5 to 2 µl per 25 µl reaction.

3. Carefully vortex and remove droplets by centrifugation.

4. Perform PCR using the recommended conditions presented below:

| Step | Temperature, °C | Incubation time | Number of cycles |
|---|---|---|---|
| Reverse transcription | 45-50 | 10-30 min | 1 |
| Preliminary denaturation | 95 | 5 min | 1 |
| Denaturation | 95 | 10 – 20 sec | 30-50 |
| Annealing | 50 – 68 (Tm-5) | 10 - 20 sec | |
| Elongation | 72 | 0.5-1 min/kbp | |

Or:

| Step | Temperature, °C | Incubation time | Number of cycles |
|---|---|---|---|
| Reverse transcription | 45 | 10-30 min | 1 |
| Preliminary denaturation | 95 | 5 min | 1 |
| Denaturation | 95 | 15 sec | 25–45 |
| Annealing/elongation | 50 – 68 | 1 min | |

5. PCR results are displayed as amplification curves.

**Optimization of reaction conditions**

1. If necessary, the reaction volume can be varied in the range of 10 to 50 µl with proportional change in the amount of all components.
2. For better passage of enzyme along the template containing GC-rich regions and regions with complicated secondary structure, the temperature can be increased to 50 °C, and/or reagents facilitating melting of the secondary structure of nucleic acids (e.g. DMSO) can be added.

**Storage conditions:** in a place protected from light at +4 ° C - 3 weeks; at -20 ° C – 1 year; no more than 50 thawing-freezing cycles.

**Transportation:** Transport in thermocontainers with cooling elements; the ambient temperature increment to the room temperature during the transportation up to 7 days is allowed.