# M-MLV III Green One-Step qRT-PCR(Low ROX) Kit

**产品信息：**

| 组成 | MT612-01<br>100×20μl rnxs | MT612-02<br>500×20μl rnxs |
|---|---|---|
| 2x Green One-Step mix(Low ROX) | 1×1ml | 5×1ml |
| M-MLV III Enzyme mix | 1×60μl | 1×300μl |
| DEPC-$H_2O$ | 1×1.8ml | 2×1.8ml |

**Storage and stability:**

**is shipped on Dry Ice and can be stored for up to 12 months at -20°C,or up to 2 weeks at 4°C. Repeated freeze/thaw cycles should be avoided.**

**Notes:**Research only

**Description：**

The M-MLV III Green One-Step qRT-PCR(Low ROX) Kit has been formulated for highly reproducible first-strand cDNA synthesis and subsequent real-time PCR in a single tube. A combination of the latest advances in buffer chemistry together with a reverse transcriptase and hot-start DNA polymerase system, ensures that The M -MLV III Green One-Step qRT-PCR(Hi ROX) Kit produces fast, highly-specific and ultra-sensitive one-step RT-qPCR.

The M-MLV III Green One-Step qRT-PCR(Low ROX) Kit consists of a 2x M -MLV III Green One-Step mix, as well as separate reverse transcriptase and RNase Inhibitor.

**PCR Reaction Conditions(for a 20μl reaction)**

| | |
|---|---|
| 2x Green One-Step mix(Low ROX) | 10μl |
| 10μM Forward Primer | 0.8μl |
| 10μM Reverse Primer | 0.8μl |
| M-MLV III Enzyme mix | 0.6μl |
| Template and Primers | as required |
| dd$H_2O$ | up to 20μl |

**PCR cycling conditions:**

The following RT-qPCR conditions are suitable for the M -MLV III Green One-Step qRT-PCR(Low ROX) Kit with the majority of amplicons and real-time PCR instruments. However, the cycling conditions can be varied to suit different machine-specific protocols.M -MLV III Green One-Step qRT-PCR(Low ROX) Kit is compatible with either 3-step or 2-step cycling:

**3-step cycling:**

| Step | Temp | Time | Cycles |
|---|---|---|---|
| Reverse transcription | 45°C | 10min | 1 |
| Polymerase activation | 95°C | 2min | 1 |
| Denaturation | 95°C | 5s | 40 |
| Annealing | 60°C | 10s | |
| Extension | 72°C | 5-20s | |

**2-step cycling:**

| Step | Temp | Time | Cycles |
|---|---|---|---|
| Reverse transcription | 45°C | 10min | 1 |
| Polymerase activation | 95°C | 2min | 1 |
| Denaturation | 95°C | 5s | 40 |
| Annealing/ Extension | 60-65°C | 20-30s | |

**Optional analysis:** After the reaction has reached completion, refer to the instrument instructions for the option of melt-profile analysis.

**The conditions above are intended as a guide only; conditions will vary from reaction to reaction and may need optimization.**

**Important considerations and optimization**

**Primers:** The sequence and concentration of the primers, as well as amplicon length, can be critical for specific amplification, yield and overall efficiency of any RT-qPCR. We strongly recommend taking the following points into consideration when designing and running your RT-qPCR:

• use primer-design software, such as Primer3 (http://frodo.wi.mit.edu/primer3/) or visual OMP™ (http://dnasoftware.com/). Primers should have a melting temperature (Tm) of approximately 60°C

• optimal amplicon length should be 80-200bp, and should not exceed 400bp

• final primer concentration of 400nM is suitable for most SYBR-Green based reactions, however to determine the optimal concentration we recommend titrating in the range 0.1-1μM

• use an equimolar primer concentration

• when possible, use of intron spanning primers to avoid amplification from genomic DNA

**Template:** It is important that the RNA template is intact and devoid of DNA or contaminating inhibitors of both reverse transcription and PCR. The recommended amount of template for one-step RT-qPCR is dependent upon the type of RNA used.

• **total RNA:** purified total RNA can be used in the range from 1pg to 1μg per 20μl reaction

• **mRNA:** purified mRNA can be used from 0.01pg per 20μl reaction

**$MgCl_2$:** The $MgCl_2$ concentration in the 1x reaction mix is 3mM. In the majority of real-time PCR conditions this is optimal for both the reverse transcriptase and the hot-start DNA polymerase. If necessary, we suggest titrating the $MgCl_2$ to a maximum of 5mM.

**RT-PCR controls:** It is important to detect the presence of contaminating DNA that may affect the reliability of the data. Always include a no-RT control, by omitting the reverse transcriptase from the reaction.

**Optional ROX:** The M-MLV III Green One-Step qRT-PCR Kit is premixed with ROX (5-carboxy-X-rhodamine, succinymidyl ester), so that where necessary, ROX fluorescence can be optionally detected on certain real-time instruments. If your real-time instrument has the capability of using ROX and you wish to use this option, then this option must be selected by the user in the software.

**Instrument compatibility**

The M-MLV III Green One-Step qRT-PCR Kit has been optimized for use in SYBR Green-based real-time PCR on the real-time instruments listed in the following compatibility table, each of these instruments having the capacity to analyze the real-time PCR data with the passive reference signal either on or off. The kit is also compatible with several instruments that do not require the use of ROX, such as the Qiagen (Corbett) Rotor-Gene™ 6000, the Bio-Rad CFX96 or the Roche LightCycler® 480.

| Manufacturer | Model |
|---|---|
| ABI | 7000, 7300, 7700, 7900, 7900HT, 7900HT FAST, StepOne™, StepOne™ Plus |



总机：010-52609502/52609503　7×12小时服务热线：15727355159
售后服务部：总机转8011/biomed168@163.com