

# USB® FidelityTaq™ RT-PCR Master Mix (2X)

- **Flexible**—FidelityTaq RT-PCR Master Mix with FidelityTaq DNA Polymerase is optimized for high-fidelity and long products.
- **Sensitive**—Targets are easily detected down to 100 fg of total RNA.
- **Long targets**—Efficiently amplify targets up to ~6 kb with the FidelityTaq RT-PCR Master Mix.
- **Convenient**—Just add RNA template and primers in one easy step.
- **Stable**—FidelityTaq RT-PCR Master Mix withstands repeated freeze-thaws with no loss in performance.

USB FidelityTaq RT-PCR Master Mix provides maximum convenience and optimal performance for highly sensitive, specific, and accurate one-step RT-PCR reactions. This unique formulation combines all the reagents necessary for successful, high-fidelity RT-PCR. Simply add FidelityTaq RT-PCR Master Mix to RNA template, primers, and RNase-free water, and the reactions are ready to begin.

During reverse transcription-polymerase chain reaction (RT-PCR), reverse transcriptase converts RNA template to cDNA which is subsequently amplified by a thermostable DNA polymerase<sup>(1, 2)</sup>.

One-step RT-PCR is a variation of RT-PCR in which the components necessary for both the RT and PCR steps are combined in a single tube and the reactions are performed sequentially<sup>(2)</sup>. This single-step, closed-tube approach simplifies the expression analyses of one or a few genes from multiple RNA samples and reduces the risk of contaminating samples. USB FidelityTaq RT-PCR Master Mix further simplifies this technique by providing high-fidelity, one-step RT-PCR in a pre-mixed format.

## Convenience

FidelityTaq RT-PCR Master Mix saves time and reduces potential contamination errors by eliminating several pipetting steps. Since the mix is pre-formulated and thoroughly QC tested, experimental variability is significantly reduced. This translates into greater reproducibility in demanding, high-throughput experiments. For a 50 µl reaction, simply add 25 µl of FidelityTaq RT-PCR Master Mix to primers, RNA template, and RNase-free H<sub>2</sub>O.

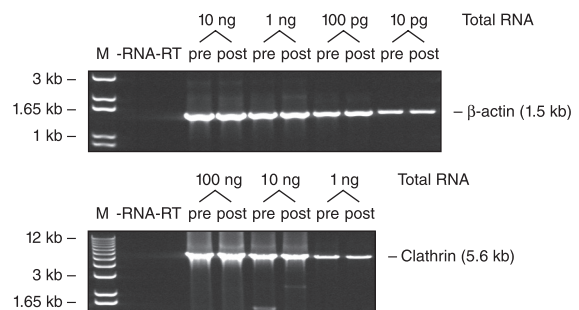
## High fidelity and longer RT-PCR products

By incorporating FidelityTaq Polymerase into the mix, amplification fidelity is increased up to 6 times over Taq Polymerase alone, which is ideal for cloning applications<sup>(3-6)</sup>. FidelityTaq RT-PCR Master Mix generates products with both blunt-ends and those with non-template added adenine on the 3' end. The ratio of blunt-ends to non-template, adenylated-ends varies from primer to primer and base identity at the 3' end<sup>(7)</sup>. Thus, RT-PCR products may be cloned into both blunt-end and TA vectors, although TA vectors may yield more transformants. In addition, longer product sizes can be generated up to about 6 kb (Fig. 1).

## Improve specificity and sensitivity

Amplify RT-PCR products from less template, with lower background, and with little or no optimization. Targets may be routinely detected from just 100 fg of total RNA. For example, human β-actin is detected from 100 femtograms of total RNA, which represents about 1/100th of the total RNA of a single human cell.

**Fig. 1. Stability and product length**



Targets were RT-PCR amplified from the indicated amounts of human total RNA. RT temperature was 50°C and primers were at 0.8 µM. The -RNA control included no RNA in the sample and the -RT control included 100 ng of total RNA but used the Taq PCR Master Mix (PN 71162, without reverse transcriptase) to test for any contaminating genomic DNA in the RNA sample. "Pre" indicates lanes in which the Master Mix was not freeze-thawed and "Post" indicates lanes in which the Master Mix was freeze-thawed 15 times alternating between dry ice and a room temperature water bath. "M" is the DNA marker lane. Human placental RNA was used for actin, and pooled RNA from several human cell-lines was used for Clathrin. The mix withstands repeated freeze-thaw cycles (up to 15) with no effect on product yield and is able to generate targets at least up to 5.6 kb.

### Optimal formulation

An enhanced buffer allows for RT reaction temperatures up to 50°C, which can improve detection of more difficult targets. This is because higher RT temperatures reduce non-specific priming and facilitate melting of RNA secondary structure<sup>(8)</sup>. In addition, the mix has the wild-type, RNase H-plus form of M-MLV Reverse Transcriptase which has been shown to improve the sensitivity for certain targets<sup>(9)</sup>. The RNase H activity degrades the RNA part of the cDNA/RNA hybrid following reverse transcription which may prevent its inhibition during subsequent PCR steps.

### Stable performance

The FidelityTaq RT-PCR Master Mix withstands repeated freeze-thaw cycles with no observed decrease in performance (Fig. 1).

### Tested User Friendly™ functional tests:

Tested by amplifying a 459 bp  $\beta$ -actin target from 10 pg and a 1.5 kb  $\beta$ -actin target from 100 pg of human placental total RNA, plus a 5.6 kb Clathrin target from 10 ng of pooled human total RNA.

### FidelityTaq RT-PCR Master Mix Formulation (2X):

Includes M-MLV Reverse Transcriptase, FidelityTaq DNA Polymerase, recombinant RNase Inhibitor, nucleotides, and magnesium in a novel RT-PCR buffer. Magnesium concentration is 3 mM in the 2X FidelityTaq RT-PCR Master Mix.

### Components:

FidelityTaq RT-PCR Master Mix is supplied in kit form with the following components sufficient for 100 reactions in a 50  $\mu$ l reaction volume:

- 4 x 625  $\mu$ l FidelityTaq RT-PCR Master Mix (2X)
- 3 x 1 ml RNase-free Water
- 1 x 1 ml 25 mM MgCl<sub>2</sub> Solution
- Protocol book and brief protocol

### Shipping and storage:

Shipped on dry ice. Store at -20°C. Mix well prior to use.

### FidelityTaq RT-PCR Master Mix (2X)

| Product code | Pack size     |
|--------------|---------------|
| 71185        | 100 reactions |

### References:

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