

Data Sheet (01.06.2005)

# mi-RT Go

Cat.-No.	Size
mi-E8101	50 rxs

**The kit for mi-RT Go contains:**

1. Reaction buffer A (5x) (MgCl<sub>2</sub> 15 mM)
2. dNTPs (5 mM)
3. Mixture of enzymes
4. DNase-RNase free water

**Storage at -20°C**

*For research use only!*

**RT Go - PCR kit**

The RT-PCR kit is especially designed for simple and effective one-tube RT-PCR. The reverse transcription step is directed by M-MuLV reverse transcriptase. As soon as the cDNA is synthesized, a chemically modified "Hot-Start" Taq DNA polymerase, is activated by heating up the reaction 10 min to 95°C. As soon as the enzyme is activated, regular PCR is performed.

**Protocol for RT Go - PCR**

please mix in the PCR-tube:

- |                           |         |                                 |
|---------------------------|---------|---------------------------------|
| 1. Total RNA              |         | 1-10 ng                         |
| 2. Reaction buffer A (5x) |         | 5 µl                            |
| 3. dNTP (5mM)             |         | 2.5 µl                          |
| 4. Primers                | reverse | 20 pmoles                       |
|                           | forward | 10 pmoles                       |
| 5. Enzymes mix            |         | 1 µl                            |
| 6. Water                  |         | up to the final volume of 25 µl |

After intensive mixing, centrifuge briefly and place the tube into the thermocycler. We recommend to use the following program:

- |      |         |                      |
|------|---------|----------------------|
| I.   | 65°C    | 5 min                |
| II.  | 4°C     | 3 min                |
| III. | 42°C    | 30 min (15 – 30 min) |
| IV.  | 95°C    | 10 min               |
| V.   | 25 - 35 | amplification cycles |
| VI.  | 72°C    | 5 min                |
| VII. | 10°C    | storage              |

Step III (reverse transcription) can be performed longer (up to 1 hour) in the case of long or highly structured template. The temperature of amplification steps should be optimized for the particular amplicon.