

SynRT Reverse Transcriptase

The most thermotolerant reverse transcriptase



CAUTION! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from synthbioenzymes.com.

The protocols and storage conditions detailed below ensure that the SynRT Reverse Transcriptase can function at its highest potential. These guidelines cover standard reaction protocol for all cDNA synthesis and unique specifications tested to provide optimal conditions for SynRT Reverse Transcriptase.

1. Product description

SynRT provides robust reverse transcriptase activity – with a 65°C optimal temperature – being the most thermotolerant reverse transcriptase in the market. The enzyme retains its highly efficient reverse transcriptase activity throughout the entire cDNA synthesis, generates the highest yields of cDNA and is also capable of synthesizing long RNA samples. SynRT is the engineered large fragment of *Bacillus stearothermophilus* DNA polymerase and it's not only capable to highly efficient full-length cDNA synthesis, but it can handle a wide range of sample types, especially the ones with secondary structures that would be hard to reverse transcribed at lower temperatures.

2. Contents

SynRT package information

| | |
|--------------|---|
| R001M | 10,000 U (200 U/μl) = 50 x 25 μl reactions Material provided: SynRT enzyme 10,000 U (50 μl), 10x SynRT Buffer (1.5 ml) and 100 mM MgSO ₄ solution (1.5 ml), 100 μM Oligo(dT) ₁₈ (60 μl), and 100 μM Random Hexamer (60 μl) |
| R001L | 40,000 U (200 U/μl) = 200 x 25 μl reactions Material provided: SynRT enzyme 40,000 U (200 μl), 10x SynRT Buffer (1.5 ml) and 100 mM MgSO ₄ solution (1.5 ml), 100 μM Oligo(dT) ₁₈ (240 μl), and 100 μM Random Hexamer (240 μl) |

3. Storage conditions for SynRT Reverse Transcriptase and Buffer

Although our experiments confirm that the SynRT Reverse Transcriptase is viable after incubation at room temperature or 4°C for 2 weeks, we recommend storing all components at -20°C to ensure its quality.

4. Guidelines for using SynRT Reverse Transcriptase and Buffer

All reaction preparations should be done over ice and in a laminar flow cabinet to attenuate cross contamination of samples. We recommend making as many mastermixes as required to reduce pipetting errors. All components of the reaction mix should be mixed and centrifuged gently.

4.1 Standard protocol for first strand cDNA synthesis

| Component | Final concentration in the 10x Mastermix |
|--|--|
| Template RNA | 1 ng to 1 µg |
| Primer (Oligo(dT) or Random Primer Mix or specific primer) | 1 µl |
| dNTP mix (10 mM each) | 1 µl |
| SynRT Buffer (5x) | 1 µl |
| SynRT Reverse Transcriptase | 1 µl |
| Nuclease-free H ₂ O | to 20 µl |

Mix gently and centrifuge. Incubate the reaction mixture for 15-60 min at 65°C. The recommended temperature is 65 °C, but the enzyme works well in broad ranges of temperature (55-70 °C).

For downstream PCR application, the volume of cDNA product should not exceed 1/10 of the PCR reaction volume.

4.2 Additional information

cDNA storage The cDNA can be used directly in PCR and qPCR or stored at -80°C. If used within one week, can be stored at -20°C.

Inactivation The enzyme can be heat inactivated by incubation at 80°C for 20 min.

Template RNA 1 ng-1 µg total RNA or 1 ng-500 ng poly(A)-RNA 1 ng-500 ng specific RNA

RNA denaturation Since SynRT works most effectively at 65°C there is no need to any initial denaturation step with GC-rich or secondary structure containing RNAs.

5. Troubleshooting

Visit our online FAQ for tips and tricks and troubleshooting information:

<https://synthbioenzymes.com/product/synrt-3/>