# OneScript® Hot Reverse Transcriptase

## Cat. No. G593

Store at -20°C.

## Product Description

**OneScript® Hot Reverse Transcriptase** is a mutational derivative of Moloney-Murine Leukemia Virus Reverse Transcriptase, that can reverse transcribe low abundance or degraded RNA, and has significantly better resistance to contaminating inhibitors such as reagents used during RNA extraction and contaminants from biological samples. High processivity and sensitivity allow for rapid cDNA synthesis of full-length cDNA fragments in a fraction of the time of leading competitors. **abm** is the only company in the world to have a reverse transcriptase engineered to offer superior cDNA synthesis performance with even the most challenging RNA samples due to its **incredible thermostability at 60-72°C**. This is the enzyme of choice for daily or demanding RNA reverse transcription. OneScript® Hot is formulated with **abm**'s RNaseOFF Ribonuclease Inhibitor offering improved resistance to oxidation compared to the high oxidation-sensitive human RNase inhibitors. RNaseOFF is stable even under very low concentrations of DTT (< 1 mM), making it the best choice for ultimate RNA protection.

| Product Component | Quantity | Part No. |
|---|---|---|
| OneScript® Hot RTase | 100 rxn (100 µl) | P104 |
| 5X RT Buffer | 400 µl | P110 |

## Additional Materials Required (not supplied)

- Primers, see Primer Selection notes
- dNTP (10 mM)
- Nuclease-free $H_2O$

## Primer Selection

- **Oligo(dT) (10 µM)** are oligonucleotides that anneal to the 3'-poly(A) + mRNA. Therefore, only mRNA or total RNA templates with 3'-poly(A) tails are used in cDNA synthesis.
- **Random Primers (10 µM)** are oligonucleotides that anneal at non-specific sites of RNA templates. Therefore, all forms of RNA can be used in cDNA synthesis.
- **Gene-Specific Primers (2 µM)** are oligonucleotides that are designed to anneal to the specific site of a target gene.



## Protocol

RT reactions should be assembled in an RNase-free environment. The use of "clean" pipettors designated for PCR and aerosol-resistant barrier tips are recommended.

1. Thoroughly thaw and mix individual components before use, and assemble reaction on ice.

| Component | Volume |
|---|---|
| 5X RT Buffer | 4 µl |
| dNTP | 1 µl |
| Primers | 1 µl |
| Total RNA or poly(A) + mRNA | Variable (1 ng - 2 µg/rxn) |
| OneScript® Hot RTase | 1 µl |
| Nuclease-free $H_2O$ | up to 20 µl |

2. Gently mix the reaction and briefly centrifuge.
3. Perform cDNA synthesis by incubating for 15 minutes at 60°C.
4. Optional: Stop the reaction by heating at 85°C for 5 minutes. Chill on ice. The newly synthesized first-strand cDNA is ready for immediate downstream applications, or for long-term storage at -20°C.

## General Notes

- Both poly(A) + mRNA and total RNA can be used for first-strand cDNA synthesis, but poly(A) + mRNA may give higher yields and improved purity of final products.
- For longer transcripts >9 kb, yields can be increased by incubating at 60°C for 30-50 minutes.
- RNA samples must be free of genomic DNA contamination.
- The ratio of Random Primers to RNA is often critical in terms of the average length of cDNA synthesized. A higher ratio of Random Primers to RNA will result in a higher yield of shorter (~500 bp) cDNA, whereas a lower ratio will lead to longer cDNA products. Due to the lower annealing temperature of Random Primers, incubate at 25°C for 10 minutes to allow for primer annealing prior to reverse transcription.
- To remove RNA complementary to the cDNA, add 1 µl of *E. coli* RNase H (Cat. No. **E018**) and incubate at 37°C for 20 minutes.

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