

Ver.22.12

## StarScript First Strand cDNA Synthesis Kit

Cat.No.ODR42

**Description:** StarScript First Strand synthesis kit contains StarScript RTase which is the mutated form of M-MLV Reverse Transcriptase, rendering the enzyme thermostable with no RNase H activity. It can retain 100% activity at 50°C; also retains more than 80% activity even at 55°C.

### Product contents

| Contents                                        | Volume                                                                       |
|-------------------------------------------------|------------------------------------------------------------------------------|
| StarScript RTase (200U/μl)                      | 10000U                                                                       |
| 5X StarScript Buffer<br>(Included 50mM DTT mix) | 200μl                                                                        |
| 10mM dNTP mix                                   | 50μl                                                                         |
| RNase free Water                                | 500μl                                                                        |
| RNase inhibitor                                 | 50μl                                                                         |
| Random hexamer (2.8nmol)                        | Lyophilized<br>Dissolve in 56μl nuclease free water to<br>obtain 50μM stock. |
| Oligo(dT) <sub>18</sub> (2.8nmol)               | Lyophilized<br>Dissolve in 56μl nuclease free water to<br>obtain 50μM stock. |

**Note:** Prepare aliquots of Oligo(dT)<sub>18</sub> and Random Hexamer to avoid multiple freeze thaw cycles.

### Storage

- Store at -20°C

### Features

|                               |                                                        |
|-------------------------------|--------------------------------------------------------|
| RNase H activity              | Negative                                               |
| Temperature of cDNA synthesis | At 42-55°C                                             |
| Synthesis length              | < 14 kb                                                |
| Synthesis Primer              | Random primer, Oligo(dT) <sub>18</sub> , Gene specific |
| Expiration date (at -20°C)    | 2 years                                                |

## Protocol

First Strand cDNA synthesis (reaction volume 20µl)

1. Prepare the following mixture in a PCR tube.

|                                             |                                                                                                                                                              |      |
|---------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------|------|
| RNA                                         | 10ng – 1µg total RNA<br>10ng – 0.5µg mRNA                                                                                                                    | - µg |
| Primer                                      | 1µl Oligo(dT) <sub>18</sub> / 1µl Random hexamer<br>or<br>0.5µl Oligo(dT) <sub>18</sub> + 0.5µl Random hexamer<br>or<br>1µl Gene-specific Primer (15 – 20µM) |      |
| dNTP (10mM)                                 |                                                                                                                                                              | 1µl  |
| Make up the volume with RNase Free Water to |                                                                                                                                                              | 14µl |

2. Heat the mixture at 65°C for 5 min and cool down immediately on ice for 1 minute. Spin down the tube by centrifugation briefly.

**Note: Use heating block or PCR machine. DO NOT use water bath.**

3. In the same tube, add the following reaction mixture:  
(Total reaction volume- 20µl)

| Reagents             | Volume |
|----------------------|--------|
| 5X StarScript Buffer | 4µl    |
| StarScript RTase     | 1µl    |
| RNase inhibitor      | 1µl    |

4. Mix gently.

**Note: For random hexamer, incubate the combined reaction mixture at 25°C for 5 min and then proceed with the next step. For oligo(dT)<sub>18</sub> or gene-specific primers, directly proceed with the next step.**

5. Incubate at 50°C for 10 min.

6. Inactivate the reaction mixture by heating at 95°C for 5 min.

**Note: Use heating block or PCR machine. DO NOT use water bath.**

7. The product can be used for PCR immediately or stored at -20°C.

**Note: As a recommendation; cDNA product should compose 10% of total reaction volume of PCR.**