

# EasyScript® One-Step gDNA Removal and cDNA Synthesis SuperMix

Cat. No. AE311

Storage: at -20°C for two years

## Description

Unique genomic DNA remover is combined with *EasyScript*® First- Strand cDNA Synthesis SuperMix to achieve simultaneous genomic DNA removal and cDNA synthesis. After cDNA synthesis, gDNA remover and reverse transcriptase are inactivated by heating at 85°C for 5 seconds.

## Highlights

- Simultaneous genomic DNA removal and cDNA synthesis in one tube to minimize RNA contamination.
- The product obtained from 15 minutes reaction is used for qPCR; the product obtained from 30 minutes reaction is used for PCR.
- cDNA up to 8 kb.

## Applications

Multiple copy gene detection

## Kit Contents

| Component                                           | AE311-02 (50 rxns) | AE311-03 (100 rxns) | AE311-04 (500 rxns) |
|-----------------------------------------------------|--------------------|---------------------|---------------------|
| <i>EasyScript</i> ® RT/RI Enzyme Mix                | 50 µl              | 100 µl              | 5×100 µl            |
| gDNA Remover                                        | 50 µl              | 100 µl              | 5×100 µl            |
| 2×ES Reaction Mix                                   | 500 µl             | 1 ml                | 5×1 ml              |
| Random Primer(N9) (0.1 µg/µl)                       | 50 µl              | 100 µl              | 5×100 µl            |
| Anchored Oligo(dT) <sub>18</sub> Primer (0.5 µg/µl) | 50 µl              | 100 µl              | 5×100 µl            |
| RNase-free Water                                    | 500 µl             | 1 ml                | 5 ml                |

## First-Strand cDNA synthesis

### 1. Reaction Components

| Component                                           | Volume               |
|-----------------------------------------------------|----------------------|
| Total RNA/mRNA                                      | 50 ng -5 µg/5-500 ng |
| Anchored Oligo(dT) <sub>18</sub> Primer (0.5 µg/µl) | 1 µl                 |
| or Random Primer (0.1 µg/µl)                        | 1 µl                 |
| or GSP                                              | 2 pmol               |
| 2×ES Reaction Mix                                   | 10 µl                |
| <i>EasyScript</i> ® RT/RI Enzyme Mix                | 1 µl                 |
| gDNA Remover                                        | 1 µl                 |
| RNase-free Water                                    | to 20 µl             |



Optional: for higher efficiency, suggest to mix RNA, primer and water first. Incubate the mixture at 65°C for 5 minutes, on ice for 2 minutes. Then add other components.

## 2. Incubation

- For anchored oligo(dT)<sub>18</sub> primer or GSP, incubate at 42°C for 15 minutes (for qPCR) or incubate at 42°C for 30 minutes (for PCR).
- For random primer, incubate at 25°C for 10 minutes. After that, incubate at 42°C for 15 minutes (for qPCR) or incubate at 42°C for 30 minutes (for PCR).

3. Incubate at 85°C for 5 seconds to inactivate enzymes.

## RT-PCR

### Reaction Components

| Component                                        | Volume   | Final Concentration |
|--------------------------------------------------|----------|---------------------|
| Template                                         | Variable | as required         |
| Forward Primer (10 μM)                           | 1 μl     | 0.2 μM              |
| Reverse Primer (10 μM)                           | 1 μl     | 0.2 μM              |
| 10× <i>TransTaq</i> <sup>®</sup> HiFi Buffer II  | 5 μl     | 1×                  |
| 2.5 mM dNTPs                                     | 4 μl     | 0.2 mM              |
| <i>TransTaq</i> <sup>®</sup> HiFi DNA Polymerase | 0.5-1 μl | 2.5-5 units         |
| Nuclease-free Water                              | Variable | -                   |
| Total volume                                     | 50 μl    | -                   |

### Thermal cycling conditions

|         |            |                |
|---------|------------|----------------|
| 94°C    | 2-5 min    | } 30-35 cycles |
| 94°C    | 30 sec     |                |
| 50-60°C | 30 sec     |                |
| 72°C    | 1-2 kb/min |                |
| 72°C    | 5-10 min   |                |

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