abbexa

## Product Manual

# One-Step gDNA Removal and cDNA Synthesis SuperMix (8 kb)

**Catalog No.:** abx098015

**Size:** 50 rxns x 20 µl / 100 rxns x 20 µl

**Storage:** Store at -20 ° C in the dark for 12 months.

**Application:** For the simultaneous removal of genomic DNA and synthesis of cDNA in one tube.

### Introduction

Complementary DNA (cDNA) is DNA synthesized from a single stranded RNA (e.g., messenger RNA (mRNA) or microRNA) template in a reaction catalyzed by the enzyme reverse transcriptase. cDNA is often used to clone eukaryotic genes in prokaryotes. The ability to synthesize DNA from an RNA template, via reverse transcription, enables researchers to study RNA with the same molecular approaches used for DNA investigations. cDNA generated by reverse transcription can be amplified using polymerase chain reaction (PCR). The combination of reverse transcription and PCR (RT-PCR) allows the detection of low abundance RNAs in a sample.

### Principle of the Assay

Genomic DNA remover is combined with a first-strand cDNA synthesis SuperMix to achieve the simultaneous removal of genomic DNA and synthesis of cDNA in one tube. This process minimizes the risk of RNA contamination. The product obtained from a 15 minute reaction can be used for qPCR, and the product obtained from a 30 minute reaction can be used for PCR. This kit can produce cDNA fragments of up to 8 kb. After cDNA synthesis, gDNA remover and reverse transcriptase are heat-inactivated at 85 ºC for five seconds.

### Kit components

1. RT/RI (Reverse Transcriptase/ Ribonuclease Inhibitor) enzyme mix: 50 µl (50 rxns), 100 µl (100 rxns)
2. gDNA remover: 50 µl (50 rxns), 100 µl (100 rxns)
3. 2x ES reaction mix: 0.5 ml (50 rxns), 1 ml (100 rxns)
4. Random primer (N9) [concentration: 0.1 mg/ml]: 50 µl (50 rxns), 100 µl (100 rxns)
5. Anchored oligo $(dT)_{18}$ primer [concentration: 0.5 mg/ml]: 50 µl (50 rxns), 100 µl (100 rxns)
6. RNase-free water: 0.5 ml(50 rxns), 1 ml (100 rxns)

### Material Required But Not Provided

1. Ice
2. Incubator(s)
3. Thermocycler (for qPCR/PCR)
4. Reaction components for qPCR/PCR including 10x High Fidelity PCR SuperMix (abx098007) and 10x High Fidelity DNA Polymerase (abx071010 or abx071011)

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## Protocol

### A. First-Strand cDNA synthesis – Reaction components

| Reaction Component | Volume |
|---|---|
| Total RNA/mRNA | 50 ng – 5 μg/ 5 ng – 500 ng |
| Anchored oligo $(dT)_{18}$ primer [concentration: 0.5 mg/ml] | 1 µl |
| or random primer [concentration: 0.1 mg/ml] | 1 µl |
| or Gene-specific primer (GSP) | 2 pmol |
| 2x ES reaction mix | 10 µl |
| RT/RI enzyme mix | 1 µl |
| gDNA remover | 1 µl |
| RNase-free water | To 20 µl |
| **Total reaction mixture** | **20 µl** |

### B. First-Strand cDNA synthesis – Assay Procedure

1. Mix the RNA, primer and water thoroughly. Incubate at 65 °C for 5 minutes and then immediately place on ice for 2 minutes. Add the other reaction components.
2. **Option 1:** For the anchored oligo $(dT)_{18}$ primer, incubate the reaction mixture at 42 °C for 15 minutes (for qPCR) or incubate the reaction mixture at 42 °C for 30 minutes (for PCR).
   **Option 2:** For the random primer, incubate the reaction mixture 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 the gDNA remover and reverse transcriptase enzymes.

### C. qPCR – Reaction components

| Reaction 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 |
| 10x High Fidelity DNA Polymerase | 0.5-1 µl | 2.5 – 5 Units |
| 2.5 mM dNTPs | 4 µl | 0.2 mM |
| 10x High Fidelity PCR SuperMix | 5 µl | 1x |
| $ddH_20$ | Variable | N/A |
| **Total reaction mixture** | **50 µl** | **N/A** |

### D. qPCR – Thermal cycling conditions

| Temperature | Time | Cycles |
|---|---|---|
| 94 °C | 2 – 5 minutes | 1 cycle |
| 94 °C | 30 seconds | 30 – 35 cycles |
| 50-60 °C | 30 seconds | |
| 72 °C | 1 – 2 kb/minute | |
| 72 °C | 5-10 minutes | 1 cycle |

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