

# HiSense™ cDNA Synthesis Master Mix (only Oligo dT)

Cat. No. CDSM-100, CDSM-200

# 1. Product Information

## Introduction

HiSense™ cDNA Synthesis Master Mix is a product formulated in an All-in-one format, allowing for more convenient and rapid synthesis of first-strand cDNA.

The Master Mix contains *M-MLV (Moloney Murine Leukemia Virus)* reverse transcriptase (RTase), ribonuclease inhibitor, dNTPs and Oligo (dT)s.

#### **Primer information**

Oligo (dT)s are oligonucleotides that anneal to the 3'-Poly(A) tail of mRNAs. The utility of Oligo (dT) is restricted to mRNA or total RNA templates with 3'-Poly(A) tails.

#### **General notes**

- 1) Both poly(A) + mRNA and total RNA can be used for firststrand cDNA synthesis, but poly(A) + mRNA may give higher yields and improved purity of final products.
- 2) RNA samples must be free of genomic DNA contamination.
- 3) To remove RNA complementary to the cDNA, add 1  $\mu$ l (2 U) of E. coli RNase H and incubate at 37°C for 20 mins.
- 4) Upon completion of the first-strand cDNA synthesis, the cDNA product can be directly applied as a template in a standard PCR/qPCR.

# 2. Contents and Storage

#### **Materials Provided**

Label	CDSM-100	CDSM-200
All-In-One 5X cDNA Master Mix	200 μl	400 μl
Nuclease free water	1 ml	2 ml

#### **Storage**

Store at -20°C

Check the label on the product for expiration date.

### 3. Test Protocol

#### Protocol

- 1) Thaw RNA templates and the All-In One 5X cDNA Master Mix on ice. Mix solutions gently but thoroughly.
- 2) Prepare the following reaction mixture in a PCR tube on ice
- 3) Mix the components well and collect by brief centrifugation. Incubate the mixture in the following reaction conditions.
- 4) The newly synthesized first-strand cDNA is ready for immediate downstream applications, or for long-term storage at -20°C.

# Reaction mixture (for 10µl reaction)

Reaction components	Volume
All-In-One 5X cDNA Master Mix	2 μl
Template RNA*	Variable
Nuclease free water	up to 10 μl
Total volume	10 μl

<sup>\*</sup>The scale of the reverse-transcription reaction can be increased as necessary. Reverse transcription of as much as 500 ng of total RNA is possible with 10  $\mu$ l of reaction solution.

#### **PCR** reaction condition

Steps	Temp(°C)	Time
Primer extension	25	5 min
cDNA synthesis*	42	15 min
Reaction Termination	85	5 sec

<sup>\*</sup>The reverse transcription time can be increased by 15 to 60 minutes or more, depending on the size of the template RNA.