



BIOLOGICAL INDUSTRIES



# EZ-First Strand cDNA Synthesis Kit For RT-PCR

Premixed solutions for the synthesis of single-stranded cDNA from RNA for use as a PCR template

Cat. No.: 20-800-50

Store at: -20°C

For long-term storage, Control RNA should be at -70°C.

## Product Description:

The Polymerase Chain Reaction (PCR) is a powerful technique for rapid amplification of genes. In addition to amplifying genomic DNA templates, PCR can also be used to amplify cDNA reverse transcribed from RNA to analyze gene expression. Using the EZ-First Strand cDNA Synthesis Kit, RNA is reverse transcribed into single-stranded cDNA. The reverse transcriptase (RT) enzyme synthesizes the new cDNA strand at a site determined by the type of primer used: Oligo(dT) primer, random primer or a sequence-specific primer.

The First Strand cDNA can then be used as a template for PCR.

The reaction mix solution supplied, contains buffer, MMLV reverse transcriptase (point mutant lacking RNase H activity), and RNase inhibitor sufficient for 50 reactions.

## Kit Reagents:

1. RT Reaction Mix	400µl
Contains: Reverse transcriptase, RNase inhibitor and dNTP's in buffer solution	
2. DTT Solution, 100mM	100µl
3. Oligo(dT) <sub>20</sub> Primer, 40µM	50µl
4. Random Hexamer Primer, 40µM	50µl
5. Control RNA	25µl
Human, Total RNA, 1µg/µl	
6. Primer Mix	50µl
Human G3PDH amplimers, 10µM each	
7. DEPC-Treated Water	1.5ml

Additional material required:

- DNA Polymerase for PCR
- Thermal Cycler
- Amplification primers specific to your target cDNA
- Microcentrifuge
- 42°C and 70°C water baths or heat blocks

## General Considerations:

1. To avoid RNase contamination and degradation of RNA, wear gloves and use sterile pipette tips.
2. High quality RNA preparation is critical for the synthesis of cDNA for PCR. RNA should have a  $A_{260}/A_{280}$  ratio of 1.7 or higher and the integrity and purity should be evaluated. The RNA should be stored at  $-70^{\circ}\text{C}$  or below.
3. cDNA Priming:

EZ-First Strand cDNA Synthesis Kit allows the user to choose the desired primer for cDNA synthesis:

- Gene specific primer: Primer design, concentration and annealing temperature should be evaluated before use.
- Oligo (dT)<sub>20</sub> or random primer: the entire population of mRNA molecules is converted into cDNA by priming with Oligo(dT) or random primer. Both primers are provided in the kit, but the use of Oligo(dT) primer is recommended. The random priming of cDNA may be beneficial when the reverse transcriptase fails to fully transcribe an mRNA template or if secondary structures exist.

## Procedure:

### 1. cDNA Synthesis

1.1 Thaw each tube in the EZ-First Strand Synthesis Kit and place on ice.

1.2 Briefly centrifuge all reagents and return to ice.

1.3 Mix the following components in a sterile, thin-walled PCR micro-centrifuge tube on ice:

RNA sample	use 0.2 – 2.0 $\mu\text{g}$ of total RNA or 50-100ng of Poly(A) <sup>+</sup> RNA	
Oligo(dT) Primer (40 $\mu\text{M}$ ) or Random Hexamer Primer	1 $\mu\text{l}$ (2 $\mu\text{M}$ )	Sequence- specific primer may be used at 2 pmol/reaction
DEPC-Treated Water	up to 10 $\mu\text{l}$	

1.4 Mix gently and heat the mixture at  $+70^{\circ}\text{C}$  for 10 minutes. Remove tube and place rapidly on ice.

1.5 Add the following components to the reaction tube:

Reaction Mix (2.5X)	8 $\mu\text{l}$
DTT (100mM)	2 $\mu\text{l}$

Mix gently by pipeting up and down.

*Note: We recommend that a master mix be prepared if more than one RNA sample is to be used.*

*Centrifuge briefly and aliquot into sample reaction tubes.*

1.6 If Random Hexamer Primer is used: incubate at  $25^{\circ}\text{C}$  for 10 minutes

1.7 Incubate the reaction tubes at  $+42^{\circ}\text{C}$  for 60 minutes.

1.8 Heat at  $+70^{\circ}\text{C}$  for 15 minutes to stop cDNA synthesis reaction. Centrifuge the mixture briefly to collect the sample at the bottom of the tube.

1.9 At this point the reaction tube, containing the cDNA, may be stored at  $-20^{\circ}\text{C}$  or below.  
For immediate use, keep tubes on ice.

## 2. PCR Amplification

2.1 Dilute the reaction mixture to a final volume of 100 $\mu$ l by adding 80 $\mu$ l of DEPC-Treated Water. Vortex gently and centrifuge.

*Note: After thawing frozen samples, vortex and spin briefly before use.*

2.2 The PCR amplification parameters will vary depending on the specific primers, template DNA and thermal cycler used.

For each 50 $\mu$ l PCR reaction, use 5-10 $\mu$ l of the diluted cDNA.

2.3 Analyze the RT-PCR product by electrophoresis in an agarose gel. Expected results will appear as a single band of size determined by the PCR primers used.

## 3. Controls

3.1 EZ-First Strand cDNA Synthesis Kit includes Control RNA and PCR Primers. For cDNA synthesis, use 1 $\mu$ l of Control RNA (human, total RNA, 1 $\mu$ g/ $\mu$ l). Then use the Primer Mix (G3PDH) for the PCR amplification.

3.2 Use the following PCR protocol for control primers and cDNA template.

### 3.2.1 PCR reaction:

- Sterile H <sub>2</sub> O	33.6 $\mu$ l
- 10X PCR Buffer	5 $\mu$ l
- dNTP's Mix (2.5mM each)	4 $\mu$ l
- Primer Mix (G3PDH)	2 $\mu$ l
- Taq DNA Polymerase (5 u/ $\mu$ l)	0.4 $\mu$ l
- Diluted cDNA	5 $\mu$ l
<b>Total:</b>	<b>50<math>\mu</math>l</b>

### 3.2.2 Amplification parameters:

- Initial Denaturation	94°C	2 minutes	} 30 cycles
- Denaturation	94°C	45 seconds	
- Annealing	60°C	45 seconds	
- Extension	72°C	2 minutes	
Final extension	72°C	7 minutes	

3.3 Upon gel electrophoresis (1.8% agarose gel), a 983 bp fragment should be observed.