Optimax First Strand cDNA Synthesis Kit

Introduction
BioChain's First Strand cDNA Synthesis Kit is developed based on our in-house cDNA synthesis protocol. The kit generates full-length first strand cDNA that is ready for PCR amplification. An easy to follow protocol is provided. The kit contains sufficient reagents for 100 cDNA synthesis reactions.

Feature
- Full-length first strand cDNA synthesis
- Ready for PCR amplification
- Templates for second strand synthesis and construction of cDNA libraries
- Complete system with positive control primers and BioChain's premium quality human placenta total RNA included

Application
- Immediate PCR amplification of known genes
- Comparison of gene expression patterns between biological samples
- Gene mutation analysis
- Gene cloning and target sequencing

Description
BioChain's First Strand cDNA Synthesis Kit provides all necessary components to perform 100 first strand cDNA synthesis reactions from total RNA or poly(A) RNA. The system uses Moloney Murine Leukemia Virus Reverse Transcriptase with weak activity of RNase H that allows the synthesis of full-length cDNA from long templates. Oligo$(dT)_{18}$ and random hexamer primers are provided. Specific primers can also be used with the kit.

The cDNA synthesized using this system can be used directly in PCR amplification. The kit is optimized for maximum yield of full-length cDNA. The addition of ribonuclease inhibitor lowers the risk of mRNA degradation during the reaction.

Content
All necessary reagents for full-length first strand cDNA synthesis are provided. There are a total of 10 vials in each kit and sufficient reagents for 100 reactions.

Quality Control
All kit components are DNase-, RNase-, and protease-free. Each component has been tested for purity and efficacy in first strand cDNA synthesis.

Storage Condition
Store all kit components at –20 °C. Product ships in dry ice.

Protocol for Synthesis of First Strand cDNA

1. Prepare template RNA, either total RNA or poly(A)+ RNA, on ice:

total RNA	2 – 6 μg
or poly (A)+ RNA	20-500 ng

2. Add primers: the primers can be either oligo(dT)18 primer, random hexamer primer or a gene specific primer:

oligo(dT)18 primer	1 μl
or/and random hexamer primer	1 μl
or gene-specific primer (50 μM)	1 μl

3. Add DEPC treated H_2O to 12 μl,
4. Mix gently and spin 5 seconds in a microcentrifuge,
5. Incubate the mixture at 65 °C for 7 min, chill on ice for 3 min,
6. Centrifuge in a microcentrifuge for 10 sec,
7. Place the tube on ice and add the following components:

5 x reverse transcriptase buffer	4 μl
10 mM dNTP	2 μl
RNasein	1 μl
MMLV reverse transcriptase	1 μl

8. Mix the reaction components by gently tap the tub several times,
9. Centrifuge 10 sec in a microcentrifuge,
10. Incubate the mixture at 42 °C for 60 to 90 min,
11. Stop the reaction by incubating the tube at 70 °C for 10 min.

Note:
1. First strand cDNA synthesized by this kit is ready for PCR amplification.
2. If the provided human placenta RNA or other human RNA samples are used, the provided primer pairs can be used to verify cDNA production and the sizes. The provided primers will amplify a region that is > 6 kb upstearm of the poly(A) tail of the mRNA. Thus, if a PCR band of 568 bp is confirmed, the cDNA sizes are up to al least 6 kb.
3. Random hexamer primer is about the same as oligo(dT)18 primer in molar concentration.

Kit Components:

Number On Cap	Component	Concentration	Amount
1	MMLV reverse transcriptase	100 unit/μl	100 μl
2	5 x Reaction Buffer	5 x	1 ml
3	Oligo(dT)18	0.5 μg/μl	100 μl
4	Random Hexamers	0.2 μg/μl	100 μl
5	RNasein	10 units/μl	100 μl
6	dNTP	10 mM each	250 μl
7	Human Placenta total RNA	1.25 μg/μl	10 μl
8	Upstream Primer	25 μM	50 μl
9	Downstream Primer	25 μM	50 μl
10	DEPC H2O		1.25 ml