For research purpose only. Not for use in diagnostic procedures for clinical purposes. For IN VITRO USE ONLY.

Power cDNA Synthesis Kit (First-strand cDNA Synthesis)

Cat. No. **25011** 30 Rxn

DESCRIPTION

The high-efficiency conversion of RNA to cDNA by the Power cDNA Synthesis Kit allows isolation and amplification of low-copy messages from small quantities of cells or tissues.
iNtRON Power cDNA Synthesis Kit is applied for a first-strand cDNA synthesis, sythesizing RNA template for PCR reaction, hence it is useful in obtaining full-length cDNA due to its high quality AMV reverse transcriptase. Degree of RNA intact in the reaction is very important for high quality cDNA synthesis and it is crucial to keep reagents from contamination such as RNase. Therefore, it is recommended to use disposable tools and reagents by treating DEPC for RNA experiments.

STORAGE AND STABILITY

Store at –20°C, under these conditions reagents are guaranteed to be stable for 12 months .

CHARACTERISTICS

- *Simple step* : The Power cDNA Synthesis protocol is optimized for speed and usability.
- *Rapid reaction time* : The Power cDNA Synthesis method can be completed within 1hour.
- *Ready-to-use* : This kit contains all the reagents required for the synthesis of cDNA, and so is very convenient.
- *High purity* : There is no RNase and DNase contamination.

CONTENTS

• AMV Reverse Transcriptase (10 U/μℓ)	15μℓ
• RT buffer (5x)	120μℓ
• Oligo $(dT)_{15}$ primer (0.2mM)	30μℓ
• Random primer (1mM)	30μℓ
• RNase inhibitor (10U/μℓ)	30μℓ
• dNTP (10mM; each 2.5mM)	60μℓ
• DTT (0.1M)	60μℓ
• DNase/RNase-free sterile water	1ml

TECHNICAL TIPS

1. *Reverse transcriptase* : All methods for synthesis of the first strand of cDNA use the enzyme RNA-dependent DNA polymerase (reverse transcriptase) to catalyze the reaction. Two different forms of reverse transcriptase are available .
2. *RNA preparation* : For high quality eukaryotic total RNA or mRNA preparations, it is necessary to minimize the activity of RNases released during cell lysis by using inhibitors Of RNases or methods that disrupt cells and simultaneously inactivate RNases. Furthermore, any contamination with RNases from other potential sources like glassware, plasticware, reagent solutions has to be avoided.
3. *Primers* : Like other DNA polymerases, avian and murine reverse transcriptases require a primer to initiate synthesis of DNA. For cloning of cDNAs, the most frequently used primer is oligo $(dT)_{12-18}$ nucleotides in length, which binds to the poly (A) tract at 3' terminus of eukaryotic cellular mRNA molecules. The random hexamer is also used in general for synthesizing the 1st strand cDNA fragments.

PROTOCOL

cDNA synthesis by the Power cDNA Synthesis Kit includes the following steps, and please avoid RNase contamination during RNA isolation steps.

1. Prepare an appropriate concentration of RNA samples, and mass up with sterile water to 9.5μℓ in RNase-free tube.
   Note : The standard reaction described below is performed in a total volume of 20μℓ for up to 1-2ng of mRNA addition or 1-2μg of total RNA.
2. Add 1μℓ of Oligo $(dT)_{15}$ or 1μℓ of Random primer, and heat for 5 min.
   Note : Heat treatment denatures RNA hairpin structure or secondary primer structure to enhance specific binding of primer and RNA. It is same reaction, heating to 65°C for 10 min.
3. Spin briefly to collect the solution at the bottom of the tube.
4. Add the following reagents and mix gently.

- RNase inhibitor	1.0μℓ
- 5x RT buffer	4.0μℓ
- dNTP	2.0μℓ
- DTT	2.0μℓ
- AMV RT enzyme	0.5μℓ

5. Incubate at 42°C in heat block (or water bath) for 60 min, and heat to 70°C for 5 min terminating the reaction.
   Note : This step is a denaturing process of RNA:cDNA hybrid.
6. Dilute the reactant above by adding 50-80μℓ sterile water into a tube containing the cDNA obtained at RT reactant.
   Note : Minimum amount of cDNA is optimal condition for PCR reaction. Therefore dilution of cDNA is recommended.
7. Proceed to PCR reaction.