ISO 9001/14001 Certified Company

Power cDNA Synthesis Kit (First-strand cDNA Synthesis)

Cat. No. 25011 30 Rxns

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's Power cDNA Synthesis Kit is applied for a first-strand c DNA synthesis, synthesizing RNA template for PCR reaction, hence it is useful in obtaining full-length cDNA due to its high quality AMV reverse transcriptase. Degr ee 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, i t is recommended to use disposable tools and reagents by treating DEPC for RNA experiments. iNtRON also provides three type of the RNA extraction Kit;

i) Solution type, easy-BLUE™ Total RNA Extraction Kit (Cat.No. 17061)
ii) Spin type, RNA-spin™ Total RNA Extraction Kit (Cat.No. 17211)
iii) Solution & spin type, easy-spin™ (DNA free) Total RNA Extraction Kit (Cat.No. 1 7221) for simple and effective extraction of RNA.

STORAGE AND STABILITY

Store at -20 °C; under these conditions reagents are stable for 12 months .

CHARACTERISTICS

- *Simple step* : The Power cDNA Synthesis protocol is optimized for speed and u sability.
- *Rapid reaction time* : The Power cDNA Synthesis method can be completed wit hin 1hour.
- *Ready-to-use* : This kit conveniently contains every required reagents for the cD NA synthesis
- *High purity* : Pre-treated to eliminate any chance of contamination with RNase and DNase.

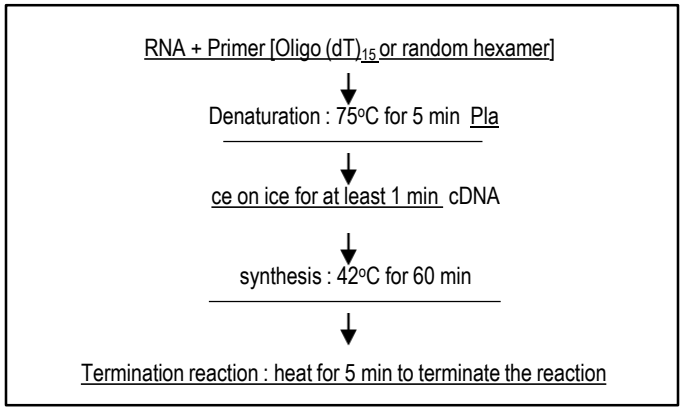
CONTENTS

• 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)	60μl
• dNTP (10mM; each 2.5mM)	60μl
• DTT (0.1M)	1ml
• DNase/RNase-free sterile water	

TECHNICAL TIPS

1. *Reverse transcriptase* : Synthesis of the first strand of cDNA requi res RNA-dependent DNA polymerase (reverse transcriptase) to cat alyze the reaction. Two different forms of reverse transcriptase are ge nerally available .
2. *RNA preparation* : For high quality eukaryotic total RNA or mRNA prepa rations, it is necessary to minimize the activity of RNases released duri ng cell lysis by using RNases inhibitors or methods that disrupt cells a nd 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 trans criptases require a primer to initiate synthesis of DNA. For cloning of c DNAs, the most frequently used primer is oligo (dT)$_{12-18}$ nucleotides in l ength, which binds to the poly (A) tract at 3' terminus of eukaryotic cell ular mRNA molecules. The random hexamer is also used in general for synthesizing the 1st strand cDNA fragments.

SCHEMATIC OF cDNA SYNTHESIS



PROTOCOL

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

1. Prepare appropriate concentration of RNA samples, and fill up with s terile water to 9.5μl in RNase-free tube.
   Note : Isolation of intact RNA is one of the important factors for synthe sis of cDNA. Therefore, it is important to optimize the isolation of RNA and prevent contamination of RNases into the RNA sample. To isolate total RNA, we recommend using easy-BLUE™ Total RNA Extraction Kit /RNA- spin™ Total RNA Extraction Kit/easy-spin™ (DNA free) Total R NA Extraction Kit. The standard reaction described below is performed in a total volume of 20μl with up to 1-2ng of mRNA or 1-2μg of total R NA.
2. Add 1μl of Oligo (dT)$_{15}$ or 1μl of Random primer, and heat to 75°C for 5 min.
   Note : Heat treatment denatures RNA hairpin structure or secondary pri mer structure to enhance specific binding of primer and RNA. Oligo (d T)$_{15}$ is recommended for priming poly(A) RNA. Random primer is suita ble primer for the detection of multiple short RT-PCR targets.
3. Spin briefly to collect the solution at the bottom of the tube.
4. Place on ice for at least 1min.
5. Add the following reagents in the order and mix gently.

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

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

TECHNICAL INFORMATION

EXPERIMENTAL INFORMATION

• *Sensitivity*

1) Result of the differentially primed RT for the first strand cDNA synthesis.
Because Oligo-dT primer and random primer are included in Power cDNA Synthesis Kit, it can be available for both total RNA and mRNA.

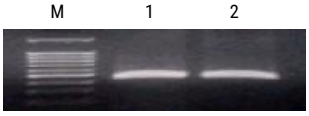


Fig. 1. RT-PCR Result by Various primers
To synthesize the first strand cDNA, both Oligo$(dT)_{15}$ primer and random primer were used separately. After dilution of each synthesized cDNA with distilled water, RT-PCR reaction were performed with GAPDH primers.
Lane M, Marker DNA; **lane 1,** oligo$(dT)_{15}$ primer; **lane 2,** random primer

2) Sensitivity for the various concentration of template RNA
Power cDNA Synthesis Kit includes the highly efficient AMV which is optimized for the purpose of synthesis of the full-length first strand cDNA. There is no alternative kit, except Power cDNA Synthesis Kit, to amplify the low copy (rare) transcripts.

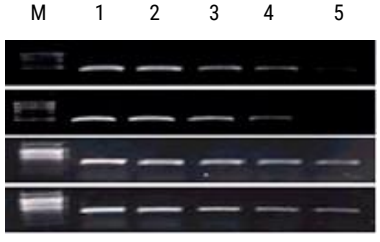


Fig. 2. Sensitivity of RT-PCR reaction with serially diluted total RNA
Total RNA were isolated from human cancer cell lines(SNU 5) using easy-BLUE™ Total RNA Extraction Kit (Cat. No. 17061). The isolated total RNA were serially diluted for RT-PCR. These results show that it can be applied for RT-PCR with small quantities of RNA.
Lane M, 100bp Ladder DNA marker; **lane 1,** No dilution template RNA; **lane 2,** 2^{-1} dilution template RNA; **lane 3,** 2^{-2} dilution template RNA; **lane 4,** 2^{-3} dilution template RNA; **lane 5,** 2^{-4} dilution template RNA

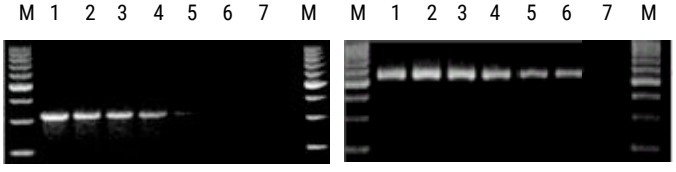
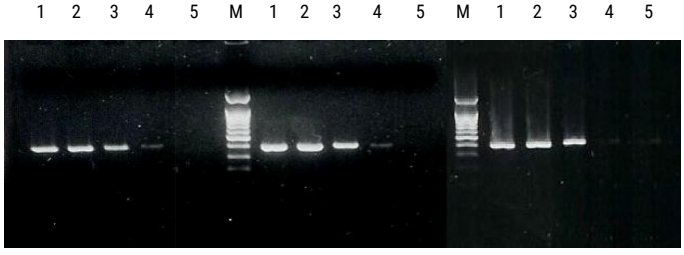


Fig. 3. Sensitivity of RT-PCR reaction with serially diluted total RNA
Total RNA were isolated from human cancer cell lines(SNU 5) using easy-BLUE™ Total RNA Extraction Kit (Cat. No. 17061). The isolated total RNA were serially diluted for RT-PCR with each two primer set (320bp and 550bp). These results show that it can be applied for RT-PCR with 100pg of template RNA at 320bp and with 10pg of template RNA at 550bp.
Lane M, 100bp Ladder DNA marker; **lane 1,** No diluted template RNA1 μg; **lane 2,** template RNA 100ng; **lane 3,** template RNA10ng; **lane 4,** template RNA 1ng; **lane 5,** template RNA 100pg; **lane 6,** template RNA10pg; **lane 7,** No insert template RNA

• Stability

1) Stability of Reverse Transcriptase (AMV and MMLV)
Power cDNA Synthesis Kit, includes the highly efficient AMV. It is suitable to synthesize the full-length cDNA and can be used at a higher temperature compared to MMLV.



42℃ rxn. **50℃ rxn.** **60℃ rxn.**

Fig. Results of RT-PCR reaction on the basis of various temperature.
Total RNA was isolated from human cancer cell lines(SNU 5) using easy-BLUE™ Total RNA Extraction Kit (Cat. No. 17061). RT-PCR were performed with β-Actin(400bp) primer set under the various temperatures. Reverse-transcription can be done at a
relatively higher temperature (60℃) than other company's.
Lane M, 100bp Ladder DNA marker; **lane 1,** template RNA 1ug; **lane 2,** template RNA 100ng; **lane 3,** template RNA 10ng; **lane 4,** template RNA 1ng; **lane 5,** template RNA 100pg

TROUBLESHOOTING GUIDE

Problem	Possible Cause	Recommendation
No PCR product or very little PCR product	Insufficient amount of template RNA	- Increase amount of RNA template in the reaction.
	Template RNA degraded	- Prepare fresh RNA template, being careful to prevent RNase activity. - Check RNA preparation by gel electrophoresis.
	Template secondary structure prevented effective first strand cDNA synthesis	-Briefly denature the RNA template at 94°C (1 min) before adding reverse transcriptase. - Caution : Do not incubate reverse transcriptase or RNase Inhibitor at this elevated temperature, as they will be inactivated.

RELATED PRODUCTS

Product Name	Cat.No.
easy-BLUE™ Total RNA Extraction Kit	17061
RNA-spin™ Total RNA Extraction Kit	17211
easy-spin™ (DNA free) Total RNA Extraction Kit	17221
Viral Gene-spin™ Viral DNA/RNA Extraction Kit	17151
ONE-STEP RT-PCR PreMix Kit!	25101