HiSenScript™ RH(-) RT PreMix Kit

The best choice of RT PreMix Kit for First-Strand cDNA synthesis work.
For highly cDNA Sensitive detection without the need for optimization.

RUO | REF 25087 | Σ 96 | -15°C -25°C

DESCRIPTION

The **HiSenScript™ RH(-) RT PreMix Kit** is designed for the sensitive and high yields synthesis and analysis of full-length cDNA copies from either a total or poly(A)+ RNA sample. The kit is optimized for a Reverse Transcriptase(RTase) reaction that can utilize any amount of total RNA from 1 pg to 5 μg per reaction and is applicable for the synthesis of full-length first strand cDNA. The RTase contained in the kit is a engineered version of REV (Reticuloendotheliosis virus; REV) RTase with reduced RNase H activity. Because HiSenScript™ RH(-) RTase is not significantly inhibited by ribosomal and transfer RNA, it may be used to synthesize first-strand cDNA from a total RNA preparation.
The RTase of the **HiSenScript™ RH(-) RT PreMix Kit** is still active at temperatures greater than 42°C. Due to its good thermal stability, formation of a stable complex of primer/template/RTase is enhanced; it is desirable for the RTase to have full activity at both a relatively high temperature and the conventional reaction temperature, which can enhance sensitivity, specificity and cDNA yield at a temperature range of 37~50°C. Specifically, it is extremely effective in the synthesis of full-length cDNA from an RNA molecule with a complicated structure that could cause difficulties in template denaturation. In addition, the RTase contained in the kit has a good inherent tolerance for inhibitory compounds, which can provide an advantage when dealing with clinical samples.

CHARACTERISTICS

- **Highest sensitivity :** can use 5 μg to 1 pg of total RNA, 500 ng to 0.01 pg of mRNA
- **Reduced RNase H activity :** increased specificity, higher yields, more first strand cDNA synthesis
- **Ready to Use :** only RNA template, Primer and RNase-free water are needed
  - ✓ All components premixed for cDNA synthesis in one tube
  - ✓ Adding template only for cDNA synthesis
- **High reproducibility test result**
- **Thermal stability :**
  - ✓ A half life of 100 min. at 42°C for the highest cDNA yields from general RNA molecules
  - ✓ A half life of 30 min. at 50°C for the highest cDNA yields from difficult or high GC-content RNA molecules.
- **Stability :** Stable for over 1 year at -20°C
- **Economic :** Time-saving and cost-effective
- **Optimized for qPCR and all types of RTase reactions**

KIT CONTENTS

Contents	Amount
HiSenScript™ RH(-) RT PreMix Kit	96 Tubes
Instruction Manual	1 ea

STORAGE AND STABILITY

- Storage condition : Store the product at -25~ -15°C after receiving.
- Expiration : **HiSenScript™ RH(-) RT PreMix Kit** can be stored for up to 12 months without showing any reduction in performance and quality under appropriate storage condition. The expiration date is labeled on the product box.

APPLICATIONS

- RNA expression study
- RT in diagnosis of pathogen
- RNA virus research
- RNA qualification study

PRODUCT WARRANTY

All products undergo extensive quality control test and are warranted to perform as described when used correctly. Immediately any problems should be reported. Satisfaction guarantee is conditional upon the customer providing full details of the problem to iNtRON within 60 days, and returning the product to iNtRON for examination.

IMPORTANT NOTES BEFORE STARTING

1) RNA Sample
- **HiSenScript™ RH(-) RT PreMix Kit** has a high tolerance for some inhibitory compounds, RNA of a high purity is important for full-length, high quality cDNA synthesis. This product is designed for use with 1 pg to 5 μg of total RNA or 0.01 pg to 500 ng of poly(A) + RNA.
- For the preparation of total RNA, we recommend using one of our products, either the easy- BLUE™ Total RNA Extraction Kit, the easy-spin™ Total RNA Extraction Kit, the easy-RED™ Total RNA Extraction Kit or the easy-RED™ BYF Total RNA Extraction Kit (the choice of the RNA Extraction Kit is dependent upon the species of the RNA and the source of the RNA).

2) cDNA Synthesis Reaction
- For difficult or high GC-content templates, set the cDNA synthesis temperature to 42~50°C.

3) cDNA Synthesis primer : random hexamers and oligo(dT) primers
- Random hexamers are the most nonspecific priming method and are typically used for difficult or high GC-content RNA. Using random hexamers, all of the RNAs in a population are templates for the first-strand cDNA synthesis.
- Oligo(dT) primers, which result in a more specific priming method, are used to hybridize to the 3' poly(A) tails, which are found in the vast majority of eukaryotic mRNAs. As poly(A) RNA constitutes approximately 1% to 2% of the total RNA, the amount and complexity of the cDNA produced is considerably less than that produced with random hexamers.

SATISFACTION GUARANTEE

At iNtRON we pride ourselves on the quality and availability of our technical support. Our Technical Service Departments are staffed by experienced scientists with extensive practical and theoretical expertise in molecular biology and the use of iNtRON products. If you have any questions or experience any difficulties regarding the **HiSenScript™ RH(-) RT PreMix Kit** or iNtRON products in general, please do not hesitate to contact us. iNtRON customers are a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at iNtRON. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques. For technical assistance and more information please call iNtRON Technical Service Department or local distributors.

QUALITY CONTROL

In accordance with iNtRON's ISO 9001 / 14001 certified Total Quality Management System, each lot of **HiSenScript™ RH(-) RT PreMix Kit** is tested against predetermined specifications to ensure consistent product quality.

Contents	Quality Control
RT Buffer, dNTP Mixture	Conductivity, pH, sterility, and performance in RT are tested.
DNase/RNase Free Water	Conductivity, pH, sterility, and performance in RT are tested. Endonuclease, exonuclease, and RNase activities are tested.
HiSenScript™ RH(-) RT PreMix	RT reproducibility assay: The RT reproducibility assay reactions are performed in using 3 batch.
Process Inspection	Accuracy of aliquot process was validated Appearance of PreMix tubes (housing, sealing contamination)

TECHNICAL ASSISTANCE

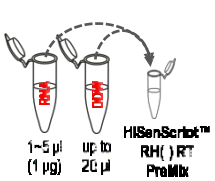
HiSenScript™ RH(-) RT PreMix Kit is intended for In vitro diagnostic medical devices. Prior to using it for other purposes, the user must validate the system in compliance with the applicable law, directives, and regulations. HiSenScript™ RH(-) RT PreMix Kit is developed, designed, and sold for in vitro diagnostic purpose. Measurement of quantities in biological samples. Metrological traceability of values assigned to calibrators and control materials.

ADDITIONAL REQUIRED EQUIPMENT

- DNase/RNase Free Water
- Pipettes and pipette tips (aerosol resistant)
- Vortex mixer
- Micro-centrifuge
- Thermal cycler
- Mineral oil (only if the thermal cycler does not have a heated lid)

QUICK GUIDE

1. Mix the Components



2. Perform RT reaction

3. Store the cDNA at -20°C or PCR analysis

PROTOCOL

1. Preparation of Reagents

1) **HiSenScript™ RH(-) RT PreMix Kit** : Leave it immediately at room temperature before use. Do not leave it at room temperature more than 1 hour.
Note : Be repeated freezing and thawing, the product may have an impact on performance.
2) **RNA** : Maintain aseptic conditions to prevent RNase contamination
3) **DNase/RNase Free Water** : No template Control (NTC)

2. RT Protocol

- Gloves are needed to wear to avoid RNA degradation.
- Leave it at 4°C or room temperature for thawing. Do not leave it at room temperature more than 1 hour.

1) **Prepare appropriate number of HiSenScript™ RH(-) RT PreMix tubes and label.**
2) **Add RNA into upper tubes.**
Note : Total RNA, 1 pg ~ 5 μg; poly(A)+ RNA, 0.01 pg ~ 500 ng
Note : (Optional) If the RNA template is GC-rich or contains secondary structures, mix gently, centrifuge briefly and incubate at 65°C for 5 min. Chill on ice and spin down.
3) **Add DNase/RNase Free Water into the tubes to a total volume of 20 μl.**

Example

cDNA Synthesis mixture	Add
Template RNA	1 ~ 5 μl
DNase/RNase Free Water	15 ~ 19 μl
Total reaction volume	**20 μl**

Note: This example serves as a guideline for RT reaction. Optimal reaction conditions such as amount of template RNA, may vary and must be individually determined.

4) **Mix the mixture well by pipetting or vortexing then spin down the mixture by brief centrifugation.**
5) **Perform RT of samples.**
Note : Suggested cycling parameters

Reaction Step	Temperature	Time
Reverse Transcription Step	42°C ~ 50°C	30 min ~ 1 hr
RTase Inactivation Step	85°C	10 min

Note : This cycling parameters serves as a guideline for RT amplification. optimal reaction conditions such as RT temperature and incubation times, may vary and must be individually determined. Recommend for difficult or high GC-content RNA, set the RT reaction to 30min at 50°C.

7) **Store the synthesized cDNA at -20°C, or proceed directly to subsequent uses such as PCR or Real-time qPCR.**

EXPERIMENT INFORMATION

1) High cDNA synthesis efficiency with difficult or high GC-content template
HiSenScript™ RH(-) RT PreMix Kit retains its thermostability at temperatures 42°C, allowing the reverse transcription to be performed at 42°C. Higher temperatures can provide higher cDNA synthesis efficiency with difficult or high GC-content RNA molecules than can RTase reactions performed at the conventional reaction temperature.

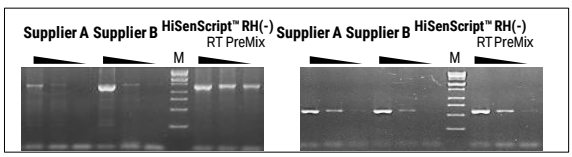


Fig. 1. Influence of temperature on cDNA synthesis efficiency for RNA molecules with complicated secondary structures such as stem-loop configurations.
Total RNA was purified from human cells (SNU-1) using an easy-spin TM Total RNA Extraction Kit (iNtRON, Cat. No.17221). The extracted RNA was serially diluted from 10 ng to 100 pg (10-fold serial dilution), and then first-strand cDNA synthesis reactions were performed using the HiSenScript™ RH(-) RT PreMix Kit under reaction temperature at 42°C. After cDNA synthesis, the 1.3kb(left; 18s rRNA) and 575 bp(right; GAPDH) of DNA fragment was amplified with the Maxime™ PCR PreMix Kit (i-Taq)(iNtRON. Cat. 21131).

2) High yield and high sensitivity cDNA synthesis efficiency
Human total RNA was serially diluted for the reverse transcription reaction, and the sensitivity of the cDNA synthesis was tested. The test result showed that the synthesis of cDNA was possible using as 1 pg to 5 μg per reaction of total RNA.

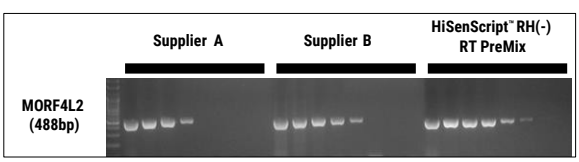


Fig. 2. Comparison of various RT premix Kit.
The cDNA synthesis was performed using serially diluted RNA using the HiSenScript™ RH(-) RT PreMix Kit. Subsequently, PCRs were performed with the synthesized MORF4L2 using a Maxime™ PCR PreMix Kit (i-Taq)(iNtRON. Cat. 21131), The human total RNA was serially diluted from 4 μg to 0.4 pg (10-fold serial dilution), and then cDNA synthesis reactions were performed RT reaction temperature at 42°C.

CAUTIONS

1. The test samples are handled under the condition of unknown level (concentration), so the laboratory contamination is expected. Therefore, all glasses used for experiments must be sterilized and secure the personal safety.
2. Always wear protective gear during handling chemical materials and the test should be handled by professionally trained person.
3. Be careful and prevent the contamination and direct contact from the test samples .
4. Centrifuge and pipette should be regularly sterilized by 10% bleach solution.
5. If there is too much RNA, several band may be shown, so the amount of RNA should be decreased.
6. All the waste should be sterilized before discarding.
7. The contamination should be considered very seriously. The work station should be kept with extreme cleanness not to have false-positive. Use RNase WiPER (iNtRON. Cat. 21131) to clean the desk or 1/20 diluted household bleach can be used alternatively.

PACKAGING INFORMATION AND STORAGE

Contents	Storage	Amount
HiSenScript™ RH(-) RT PreMix Kit	-25 ~ -15°C	96 tubes

SHELF-LIFE

- 12 months from manufacturing date.
- Within 3 months after opening, within expiry date of the kit.

EXPLANATION OF SYMBOLS

LOT Batch number	RUO Research Use Only	REF Product number
Σ Sufficient for tests	Do not reuse	Storage temperature limitation
Manufacturing date	Expire date	Keep away from sunlight
Manufactured by	Keep dry	Consult Instructions For Use
EC REP Authorized Representative in European Union		Attention

TROUBLESHOOTING GUIDE

Observation	Possible Cause	Recommendation
Little or no RT reaction product	Pipetting error or missing reagent	Repeat the RT. Check the concentrations and storage conditions of the kit and template RNA.
	Problems with starting template	Check the concentration, storage conditions, and quality of the starting template. If necessary, make new serial dilutions of template nucleic acid from stock solutions. Repeat the RT using the new dilutions
	Procedural error in first-strand cDNA synthesis	Repeat the procedure carefully.
	Insufficient mixing of reaction master mix during vortexing	Vortex tube thoroughly. It is important that the dried material contained in each tube is sufficiently dissolved with template solution and D.W.
	Volume of reverse-transcription reaction added to the subsequent PCR was too high	Adding an excessive volume of reverse-transcription reaction to the PCR mix may reduce PCR efficiency. Generally, the volume of reverse-transcription reaction added to the subsequent PCR should not exceed 10% of the final PCR volume.
	Pipetting error	Check the pipette first to minimize pipetting error. Calibration is essential to ensure correct pipette operation. Repeat the preparation of reaction mix using a correct pipette.
	RNase contamination	Maintain aseptic conditions to prevent RNase contamination. Spray and wipe out the contaminent with RNase WiPER™(iNtRON, Cat. No. 21131) on your experimental surface.
RT-PCR Product bands are Short length or smeared	Too high incubation temperature	Reverse-transcription reaction should be carried out at temperature lower than 50°C. Higher temperatures than 50°C may reduce the length of cDNA products. Check the actual temperature of your heating block or water bath
	Heat inactivation	In analysis of long cDNAs, heat inactivation of HiSenScript™ RH(-) Reverse Transcriptase is not recommended, which may cause the reduced amount of full-length cDNA template resulting in a shorter PCR product than expected.

Technical advice : +82-505-550-5600

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