HiSenScript™ RH(-) RT-PCR PreMix Kit

The best choice of RT-PCR PreMix for one step RT-PCR work.
For highly sensitive detection without the need for optimization.

RUO | REF 25135 | Σ 96 | -15°C ~ -25°C

DESCRIPTION

The **HiSenScript™ RH(-) RT-PCR PreMix Kit** is designed for high sensitive and reproducible detection and analysis of low copies from either a viral RNA or RNA molecules by RT-PCR. Both reverse transcription and PCR are performed in a single tube using target gene specific primer/probe and template RNAs from either total RNA or mRNA. The reaction conditions for **HiSenScript™ RH(-) RT-PCR PreMix Kit** have been optimized to support a wide range of RT-PCR applications including real-time quantitative procedures.

HiSenScript™ RH(-) RT-PCR PreMix Kit is a dried mixture of engineered version of REV (Reticuloendotheliosis virus; REV) reverse transcriptase that reduced RNase H activity and recombinant DNA polymerase complexes with proprietary hot-start specific antibody that inhibits polymerase activity to synthesize DNA from an RNA template. HiSenScript™ RH(-) Reverse Transcriptase can synthesize cDNA at a temperature range of 40–50°C, providing increased specificity, more full-length product than other reverse transcriptase. i-StarMAX™ GH DNA polymerase activity is restored after the denaturation step in PCR cycling at 94°C, providing an automatic "hot start" in PCR for increased sensitivity, specificity, and yield. The kit included in consists of a proprietary buffer system that has been optimized for reverse transcription and PCR, Mg^{2+}, dNTPs, tracking dye and stabilizers. The convenient 2X format allows you to add template and primer at any desired concentration.

CHARACTERISTICS

- **Highest sensitivity :** can use less than 1 pg of total RNA, 0.1pg of mRNA
- **Reduced RNase H activity :** increased specificity, higher yields, more first strand cDNA synthesis
- **Ready to Use :**
  - ✓ All components premixed for RT-PCR in one tube
  - ✓ Adding RNA template and Primer areneeded
- **High reproducibility test result**
- **Thermal stability:**
  - ✓ A half life of 100 min. at 45°C for the highest cDNA yields from general RNA molecules
  - ✓ Can be used with highly structured RNA or gene-specific primers
- **Stability :** Stable for over 1 year at -20°C
- **Economic :** Time-saving and cost-effective
- **Optimized for all types of RT-PCR reactions**

KIT CONTENTS

Contents	Amount
HiSenScript™ RH(-) RT-PCR PreMix	96 Tubes (12 strips)
Instruction Manual	1 ea

STORAGE AND STABILITY

- Storage condition : Store the product at -25 ~ -15°C after receiving.
- Expiration : **HiSenScript™ RH(-) RT-PCR 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-PCR 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-PCR 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 1 μg of total RNA or 0.01 pg to 100 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) RT-PCR Reaction
- RNA should be devoid of any RNase contamination and aseptic conditions should be maintained.

3) RT-PCR primers
- We recommend using gene-specific primer (GSP). We do not recommend using oligo (dT) or random primers, because they can generate nonspecific products in the one-step procedure and the amount of RT-PCR product may be reduced.
- A final primer concentration of 0.2 μM for each primer is generally optimal. However, for best results, we recommend performing a primer titration of 0.15 ~ 0.5μM.

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-PCR 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-PCR PreMix Kit** is tested against predetermined specifications to ensure consistent product quality.

Contents	Quality Control
RT-PCR Buffer, dNTP Mixture	Conductivity, pH, sterility, and performance in RT-PCR are tested.
DNase/RNase Free Water	Conductivity, pH, sterility, and performance in RT-PCR are tested. Endonuclease, exonuclease, and RNase activities are tested.
HiSenScript™ RH(-) RT-PCR PreMix	RT-PCR reproducibility assay: The RT-PCR 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

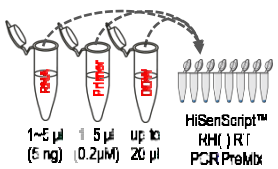
HiSenScript™ RH(-) RT-PCR PreMix Kit is intended for In vitro diagnostic medical devices. Prior to using it for other purposes, the user must validate the system in compliance with the applicable law, directives, and regulations. **HiSenScript™ RH(-) RT-PCR PreMix Kit** is developed, designed, and sold 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

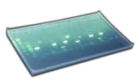
1. Mix the Components



2. RT-PCR run

3. Electrophoresis

4. Data analysis



PROTOCOL

1. Preparation of Reagents

1) **HiSenScript™ RH(-) RT-PCR PreMix** : 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-PCR 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-PCR PreMix tubes and label.**
2) **Add RNA into upper tubes.**
Note : Total RNA, 1 pg ~ 1 μg; poly(A)+ RNA, 0.1 pg ~ 100 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, spin down and place the tube back on ice.
3) **Add DNase/RNase free water into the tubes to a total volume of 20 μl.**

Example

RT-PCR mixture	Add
Template RNA	1 ~ 5 μl
Primer (F : 10pmol/μl)	1μl
Primer (R : 10pmol/μl)	1μl
DNase/RNase Free Water	13 ~ 17 μl
Total reaction volume	**20 μl**

Note: This example serves as a guideline for RT-PCR 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-PCR of samples.**
Note : Suggested cycling parameters

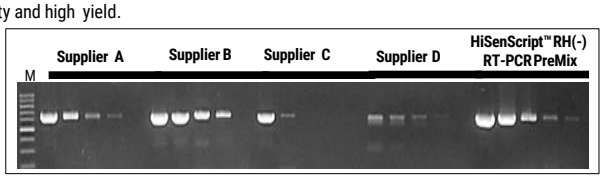
RT-PCR cycle		Temp.	RT-PCR product size		
			100-500bp	500-1000bp	1kb-5kb
1 Cycle	RT	45℃~55℃	30min	30min	60min
1 Cycle	Denature	94℃	5min	5min	5min
30-40 Cycles	Denaturation	94℃	20 sec	20sec	20sec
	Annealing	50-65℃	20 sec	20sec	30sec
	Extension	65-72℃	20-30 sec	40-50sec	1min/kb
1 Cycle	Final extension	72℃	Optional. Normally, 2-10 min		

Note : This cycling parameters serves as a guideline for RT-PCR amplification. optimal reaction conditions such as RT-PCR temperature and incubation times, may vary and must be individually determined.

6) Load samples on agarose gel without adding a loading-dye buffer and perform electrophoresis.

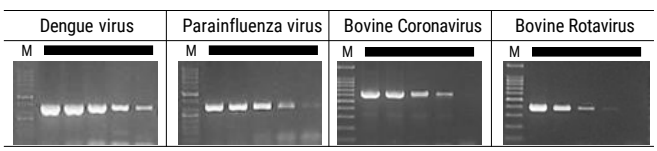
EXPERIMENT INFORMATION

1) High yield and reproducibility
HiSenScript™ RH(-) RT-PCR PreMix Kit shown enhanced RT-PCR efficiency in more sensitivity and high yield.



Total RNA from cultivated human cell (SNU-1) was used as RT-PCR template from 10 ng to 1 pg (GAPDH gene, 1/10 serial dilution).

3) Applied to Diagnostic Products
HiSenScript™ RH(-) RT-PCR PreMix Kit allows fast and easy RT-PCR setup. Whatever the application (virus detection or molecular diagnostics research) just mix all components together in one tube and start the thermal-cycler program. The reaction mixture contains all of the reagents required for both reverse transcription and PCR; nothing needs to be added once the reaction has been started.



Viral genome RNA was used as RT-PCR template from 10^{-2} to 10^{-6} (1/10 serial dilution).

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.
8. Store the kit at -20°C.

PACKAGING INFORMATION AND STORAGE

Contents	Storage	Amount
HiSenScript™ RH(-) RT-PCR PreMixKit	-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
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-PCR reaction product	Pipetting error or missing reagent	Repeat the RT-PCR. 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-PCR using the new dilutions
	Procedural error in RT-PCR	Repeat the procedure carefully.
	Insufficient mixing of reaction master mix during vortexing	Vortex tube thoroughly. It is important that the dried PreMix material contained in each tube is sufficiently dissolved with template solution and D.W.
	RT-PCR inhibitors are present in RNA	Remove inhibitors in the RNA preparation by an additional 70% ethanol wash. Inhibitors of RT-PCR include SDS, EDTA, guanidium salts, formamide, sodium phosphate and spermidi.
	Cycle number is too low	Increase cycle number.
	Extension time is too short	Set extension time for at least 60 seconds per kb of target length.
	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	RT-PCR 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
	Contamination by genomic DNA	Pretreat RNA with DNase I, Amplification Grade, as described in the DNase I documentation. Design primers that anneal to sequence in exons on both sides of an intron or at the exon/exon boundary of the mRNA to differentiate between amplified cDNA and potential contaminating genomic DNA.

Technical advice : +82-505-550-5600

Review date : 2015. 03. 27