

## NOVASCRIPT III SINGLE STEP RT-PCR SYSTEM

Cat # NS-SS-RTPCR-25R

25 Reactions

A robust, one step -simple & sharp 2x RT-PCR mix optimized for as little as 100pg containing Novascript III RNase H<sup>-</sup> Reverse Transcriptase and AuPreP Hot start Polymerase

### Product Features

- Simple to use! one-step set-up
- 2x RT-PCR Mix optimized for as little as 100pg Total RNA
- Contains a highly sensitive blend of Novascript III Reverse Transcriptase and AuPreP Hot-StartPolymerase-
- Supplied with a dNTP containing buffer
- Highly optimized for outstanding results

### Applications

- Quantitative PCR
- Gene expression analysis
- Gene cloning

### Description

NOVASCRIPT III SINGLE STEP RT-PCR SYSTEM has been designed for highly sensitive one-step RT-PCR reactions using any RNA template. The Kit employs an enzyme formulation, which includes AuPreP Hot-Start Polymerase and Novascript our reverse transcriptase that possesses low RNase H activity and is highly sensitive even when the amount of template is a limiting factor. The Kit provides highly specific reverse transcription and PCR in a single tube, using gene-specific primers on either total RNA or mRNA. The kit is provided with RNase inhibitor to protect template RNA from degradation. The special buffer is highly optimized and balanced, leading to outstanding results. The Kit is ideal for the synthesis of double-stranded cDNA products for subsequent cloning, sequencing, expression, or transcription analysis.

The Kit can be used with starting amounts of RNA template from 100pg to 2µg. After cDNA synthesis has been performed, the reaction is heated to 95°C for 10 minutes to inactivate Novascript III, and simultaneously to activate AuPreP Hot-Start DNA Polymerase (included). AuPreP Hot-Start DNA Polymerase improves specificity by eliminating the presence of non-specifics, primer-dimers, and mis-primed products.

### Storage Conditions:

NOVASCRIPT III SINGLE STEP RT-PCR SYSTEM can be stored for one year at -20°C.

### Shipping Conditions:

On Dry Ice or Blue Ice

### Components:

NOVASCRIPT III SINGLE STEP RT-PCR SYSTEM components:

|  |              |
|--|--------------|
| NOVASCRIPT III SINGLE STEP RT-PCR SYSTEM | 25 Reactions |
| Enzyme Mix                               | 50µl         |
| 2x One-Step RT-PCR Reaction Buffer       | 625µl        |
| RNase Inhibitor (10u/µl)                 | 25µl         |
| 50mM MgCl <sub>2</sub> Solution          | 1.2ml        |
| DEPC-treated Water                       | 1.2ml        |

## NOVASCRIPT III SINGLE STEP RT-PCR SYSTEM - Reaction Guidelines

### Template Quality

- Intact, high-quality RNA is essential for the reverse-transcription reaction
- All reagents for use with RNA must be prepared using DEPC-treated Water
- The inclusion of an RNase Inhibitor protein can reduce template degradation and increase yield of PCR product
- Low-copy-number genes may require an increase in starting material
- It is necessary to use a suitable RNA extraction reagent e.g., EZ RNA ISOLATION REAGENT

### Primer Design and Concentration

- The use of gene-specific primers is recommended for use with the **NOVASCRIPT III SINGLE STEP RT-PCR SYSTEM**. The use of oligo dT or random hexamers is not recommended with a One-Step RT-PCR set-up since this can result in the generation of non-specific products
- In most cases a final primer concentration of 200nM is sufficient. However, we recommend a primer titration within the 50-500nM range
- Primers should be checked to ensure that they are not self-complementary
- Primer design can benefit from the use of an RNA secondary structure prediction model (e.g. MFOLD), to ensure that priming is not prevented by internal double-stranded regions caused by folding
- The use of intron-spanning primers allows differentiation between amplified cDNA and contaminating genomic DNA
- Annealing temperature of primers is usually melting temperature ( $T_m$ ) minus 5-10°C .

### MgCl<sub>2</sub> Optimization

- The final reaction will contain 1.5mM MgCl<sub>2</sub> (the 2x One-Step RT-PCR buffer contains 3mM MgCl<sub>2</sub>), which should be optimal for most reverse transcriptase and PCR reactions
- MgCl<sub>2</sub> requirements for the reaction can vary, depending on the particular template and primers used
- A titration of MgCl<sub>2</sub> can be performed to optimise the reaction conditions
- The table below shows how much of the 50mM MgCl<sub>2</sub> solution (provided) should be added to each reaction to provide an elevated concentration in the final reaction

| Volume of 50mM MgCl <sub>2</sub> to be added to a 50µl reaction | Final concentration of MgCl <sub>2</sub> |
|---|--|
| 0   | 1.5mM                                    |
| 0.5µl   | 2.0mM                                    |
| 1.0µl   | 2.5mM                                    |
| 1.5µl   | 3.0mM                                    |
| 2.0µl   | 3.5mM                                    |
| 2.5µl   | 4.0mM                                    |
| 3.0µl   | 4.5mM                                    |
| 3.5µl   | 5.0mM                                    |
| 4.0µl   | 5.5mM                                    |

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## NOVASCRIPT III SINGLE STEP RT-PCR SYSTEM -- Protocol

1) Assemble the following components on ice in a certified RNase-free reaction tube:

| COMPONENT                            | VOLUME (µl)  | FINAL CONCENTRATION                     |
|--------------------------------------|--|---|
| 2x One-Step RT-PCR Buffer (supplied) | 25   | 1x                                      |
| One-Step Enzyme Mix (supplied)       | 2  | -                                       |
| Forward Primer (5µM)                 | 2  | 200nM                                   |
| Reverse Primer (5µM)                 | 2  | 200nM                                   |
| RNA Sample                           | 1-10   | User-determined (100pg-2µg recommended) |
| Rnase Inhibitor (supplied)           | 1  | 10 Units                                |
| MgCl <sub>2</sub> (supplied)         | 2x Reaction Buffer contains 3mM MgCl <sub>2</sub> .<br>However additional Mg <sup>2+</sup> may be required (see reaction guidelines) | 1.5mM<br>(Unless adjusted by the user)  |
| DEPC-Treated Water (supplied)        | Up to final volume of 50µl   | -                                       |
| <b>Total Volume 50µl</b>             |  |   |

2) Program the Thermal Cycler to include the RT and subsequent PCR step:

3) 1 cycle of:

| Temperature | Duration      | Comments   |
|-------------|---------------|--|
| 37-45°C     | 15-30 minutes | We recommend that initial reverse-transcription steps are carried out for 30 minutes at 42°C (see reaction guidelines) |
| 95°C        | 10 minutes    | To denature RT enzyme and activate DNA Polymerase  |

4) Mix reactions gently, load into thermal cycler and start reaction.

5) Analyze the amplified product.

### RT-PCR Troubleshooting Guide

| Observation       | Possible Cause                                 | Recommended solution(s)   |
|-------------------|--|---|
| No cDNA synthesis | RNA Degraded:                                  | Analyze RNA on a denaturing gel to verify integrity. Ensure that all reagents are RNase-free.   |
|                   | RNA contained an RT inhibitor:                 | The presence of inhibitors can be determined by mixing a control RNA with some of the sample and comparing the yield with that of the original amplification. Remove inhibitors such as SDS, EDTA, formamide and pyrophosphate, by ethanol precipitation of RNA, including a 70% ethanol wash step. |
|                   | Reaction temperature not optimal:              | Perform a temperature-gradient experiment.  |
|                   | Not enough starting RNA:                       | Increase the amount of starting RNA; this can be an important factor when amplifying low-copy genes from total RNA.   |
|                   | RNA had high secondary structure:              | Prior to reaction set-up, denature RNA with primers. Raise the temperature of the RT step, up to a maximum of 60°C (for short amplicons).   |
|                   | Target not expressed in tissue analyzed:       | Try a different target of tissue.   |
| Poor Specificity  | Non-specific annealing of primers to template: | Use gene-specific primers rather than Oligo dT or random hexamers.<br>Increase the annealing temperature.<br>Increase the T <sub>m</sub> of the primers.<br>Check for presence of pseudogenes.<br>Set up reactions on ice.  |

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| Observation | Possible Cause             | Recommended solution(s)                     |
|-------------|----------------------------|---|
|             | Primer dimmers:            | Redesign primers to prevent self-annealing. |
|             | Genomic DNA contamination: | Try a different target of tissue.           |

## Suggested Controls

Performing the following controls may prove useful in validating your results.

- Control for DNA contamination (no RT control):** Set up a standard **NOVASCRIPT II SINGLE STEP RT-PCR SYSTEM** reaction and start at the 10 min 95°C step (to denature RT and activate DNA Polymerase) followed by normal PCR cycling for 40 cycles:

| Temperature | Duration                   | Comments  |
|-------------|----------------------------|---|
| 95°C        | 30 seconds                 | Template denaturation   |
| 50-60°C     | 30 seconds                 | Primer annealing (actual temperature determined by primer sequence, see guidelines) |
| 72°C        | 15-30 seconds per kilobase | Extension step  |

| <u>Other AuPreP™ DNA/RNA Kits</u>                                 | <u>Other Related Products</u>   |
|---|---|
| <b>AuPreP™ Plasmid Maxi Kit</b>                                   | <b>AuPreP Oligos</b> (High Affinity Purified Oligo synthesis available in different scales, purifications & modifications)                                  |
| <b>AuPreP™ Plasmid Midi Kit</b>                                   | <b>AuPreP TaQ DNA Polymerase</b> (Ultrapure, Ultra-stable & Ultra-sensitive Taq DNA Polymerase)   |
| <b>AuPreP™ SPIN™ SPIN Miniprep Kit</b>                            | <b>AuPreP Hotstart TaQ DNA Polymerase</b> (Robust Polymerase for Hotstart PCR assays)   |
| <b>AuPreP™ Blood Genomic DNA Maxi</b>                             | <b>AuPreP Super Fidelity TaQ DNA Polymerase</b> (High fidelity Polymerase produces blunt ended amplicons upto 5Kb)  |
| <b>AuPreP™ Blood Genomic DNA Extraction Midi Kit</b>              | <b>PCR Doctor</b> - (PCR enhancer for AuPreP Hotstart Taq or Super Fidelity Taq especially designed for GC/AT/Dirty/Difficult Templates)                    |
| <b>AuPreP™ GEN<sup>bt</sup> DNA Extraction Kit</b>                | <b>AuPreP Longjump Polymerase</b> (Robust Long Polymerase for templates > 4kb to 18kb+ for challenging PCRs)  |
| <b>AuPreP™ DNA easy Plant Maxi kit</b>                            | <b>AuPreP Red PCR Master Mix</b> ( 2x Master mix with Red Dye without Enhancer)   |
| <b>AuPreP™ DNA easy Plant Mini Kit</b>                            | <b>AuPreP DIAMOND MASTER-MIX</b> (2x Mastermix with PCR Enhancer & Stabilizer without tracking dyes)  |
| <b>AuPreP™ PCR Purification Kit</b>                               | <b>AuPreP DIAMOND DOUBLE DYE MASTERMIX</b> (2x Mastermix with PCR Enhancer, Stabilizer & tracking dyes)   |
| <b>AuPreP™ Plant RNA Maxi Kit</b>                                 | <b>AuPreP DNA Extraction System</b> ( A fast Reagent for pure genomic DNA isolation for down stream applications )  |
| <b>AuPreP™ Plasmid Maxi Kit</b>                                   | <b>AuPreP RNA Extraction System</b> ( for Purest & High Quality RNA extraction with simple cost effective protocol )  |
| <b>AuPreP™ RNA Easy Midi Kit</b>                                  | <b>AuPreP Gold cDNA Synthesis Kit</b> (Highly Cost effective cDNA Synthesis Kit using RT with reduce Rnase H activity)                                      |
| <b>AuPreP™ RNA<sup>tm</sup> Mini Kit</b>                          | <b>AuPreP Gold RT-PCR Combo Kit</b> ( 2 step RT-PCR protocol with tracking Dye )  |
| <b>AuPreP™ RNV<sup>tm</sup> Viral RNA Extraction Miniprep Kit</b> | <b>AuPreP Extra Mile First Strand cDNA System</b> ( Premium cDNA Synthesis Kit using RT with point mutant Rnase H minus activity )                          |
|   | <b>Novascript III RNase H<sup>-</sup> RT</b> (Premium Ultra-stable Rnase H minus RT for long high quality cDNA construction )                               |
|   | <b>Novascript III single step RT-PCR System</b> ( Premium 1step RT-PCR system using Novascript & AuPreP Hotstart DNA Polymerase)                            |
|   | <b>AuPreP Random Primer labeling Mix System</b> ( Premixed solution for the labeling of DNA with radiolabeled dCTP using random sequence oligonucleotides ) |