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AuPreP Citations

AuPreP Extra Mile First Strand cDNA System

AuPreP Extra Mile First Strand cDNA System is a high quality, robust, stable and optimized premixed system with RNase H minus Reverse Transcriptase including the requisite components of a cDNA assembly system. The synthesized cDNA is of very high quality and length, for your onward applications viz. PCR, cDNA libraries

Cat. No. : AUP-EXM-50 **Storage: - 20°C** **Shipping Conditions: Dry Ice**

System Details:

AuPreP Extra Mile First Strand cDNA System is a stable, high quality system with RNase H minus Reverse Transcriptase (point mutant lacking RNase H activity). Using this powerful system, RNA is reverse transcribed from RNA into single stranded cDNA, which further, can be amplified by the Polymerase Chain Reaction (PCR) to analyze gene expression. The site of the new cDNA strand synthesized by Reverse Transcriptase (RT) enzyme is determined by the type of primer used by the user viz. Oligo (dT) primer, random primer, or a sequence-specific primer. The synthesized cDNA is of very high quality and length and can then be used as a template for PCR.
The Extra Mile mix supplied in the system contains buffer, Point mutant RNase H minus reverse transcriptase, and RNase inhibitor sufficient for 50 reactions.

Salient Features:

→ **AuPreP Extra Mile First Strand cDNA System produces very high quality and long cDNA fragments, which shows excellent performance in RT-PCR experiments and cDNA library construction.**

→ **AuPreP Extra Mile First Strand cDNA System shows excellent performance with sequence specific primers, Oligo (dT) primers as well as with random primers.**

→ **AuPreP Extra Mile First Strand cDNA System shows superior performance due to RNase H minus Reverse Transcriptase and very high quality ultra pure**

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System Components

The following components are supplied with the AuPreP Extra Mile First Strand cDNA System:

Extra Mile Mix Contains; Reverse transcriptase, RNase inhibitor and >99% pure dNTP's in buffer solution	400µl
DEPC-Treated Water	1.5ml
Random Hexamer Primer, 40µM	50µl
Primer Mix Human G3PDH amplimers, 10µM each	50µl
Oligo $(dT)_{20}$ Primer, 40µM	50µl
DTT Solution, 100mM	100µl
Control RNA Human, Total RNA, 1µg/µl	25µl

NB: *Material not supplied with the system include PCR components viz. Amplification primers specific to your target cDNA, DNA Polymerase for PCR, Thermal Cycler, Microcentrifuge, 42ºC and 70ºC water baths or heat blocks.*

Important Notes

Please read the notes below before starting the procedure.

- *Wear gloves and use sterile pipette tips to avoid RNase contamination and degradation of RNA.*
- *High quality RNA preparation is critical for the synthesis of cDNA for the synthesis of cDNA for PCR. RNA should have a A_{260}/A_{280} ratio of 1.7 or higher and the integrity and purity should be evaluated. The RNA should be stored at -70ºC or below.*
- *cDNA Priming:* ***AuPreP Extra Mile First Strand cDNA System*** *allows the user to choose the desired primer for cDNA synthesis.*
    1. *Oligo $(dT)_{20}$ or random primer: the entire population of mRNA molecules is converted into cDNA by priming with Oligo (dT) or random primer. Both primers are provided in the kit, but the use of Oligo (dT) primer is recommended. The random priming of cDNA may be beneficial when the reverse transcriptase fails to fully transcribe an mRNA template or if secondary structures exist.*

2. *Gene Specific Primer: Primer design, concentration and annealing temperature should be evaluated before use.*

Flow Chart Procedure for cDNA Synthesis

Thaw all the tubes of AuPreP Extra Mile First Strand cDNA System and place on ice.

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Briefly centrifuge all the reagents and return to ice.

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Mix the following components in a sterile, thin-walled PCR micro-centrifuge tube on ice

RNA Sample ---------- use 0.2 – 2.0µg of total RNA or
50-100ng of Poly $(A)^+$ RNA.

Oligo (dT) Primer (40µM) ------- 1µl (2µM)
Random Hexamer Primer -------- 1µl (2µM)
} Sequence-specific primer may be used at 2 pmol/reaction.

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Gently mix and heat the mixture at 70ºC for 10 minutes. Remove tube and place rapidly on ice.

↓

Add the following components to the reaction tube.

Reaction Mix (2.5X) ---------- 8µl
DTT (100 mM) ---------- 2µl

Mix Gently by pipetting up and down.

Note: *We recommend that a master mix be prepared if more than one RNA sample is to be used. Centrifuge briefly and aliquot into sample reaction tubes.*

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If Random Hexamer Primer is used: incubate at 25ºC for 10 minutes.

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Incubate the reaction tubes at 42ºC for 60 minutes.

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Heat at 70ºC for 15 minutes to stop cDNA synthesis reaction. Centrifuge the mixture briefly to collect the sample at the bottom of the tube.

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At this point the reaction tube, containing the cDNA, may be stored at - 20º or below. For immediate use, keep tubes on ice.

Subsequent Suggested Protocol

1. For PCR Amplification:

→ Dilute the reaction mixture to a final volume of 100µl by adding 80µl of DEPC-Treated Water. Vortex gently and centrifuge.
Note: *After thawing frozen samples, vortex and spin briefly before use.*

→ The PCR amplification parameters will vary depending on the specific primers, template DNA and thermal cycler used.
For each 50µl PCR reaction, use 5-10µl of the diluted cDNA.

→ Analyze the RT-PCR product by electrophoresis on an agarose gel. Expected results will appear as a single band of size determined by the PCR primers used.

2. Controls:

→ AuPreP Extra Mile First Strand cDNA System includes Control RNA and PCR Primers. For cDNA synthesis, use 1µl of Control RNA (human, total RNA, 1µg/µl). Then use the Primer Mix (G3PDH) for for the PCR amplification.

→ Use the following PCR protocol for control primers and cDNA template.

- **PCR reaction**

Sterile H_2O ----------	33.6µl
10X PCR Buffer ----------	5µl
dNTP's Mix (2.5mM each) ----------	4µl
Primer Mix (G3PDH) ----------	2µl
Taq DNA Polymerase (5u/µl) ----------	0.4µl
Diluted cDNA ----------	5µl
Total:	**50µl**

- **Amplification Parameters**

Initial Denaturation---------94ºC----------	2 minutes	
Denaturation----------94ºC----------	45 seconds	30 cycles
Annealing----------60ºC----------	45 seconds	
Extension----------72ºC----------	2 minutes	
Final Exension----------72ºC----------	7 minutes	

→ Upon gel electrophoresis (1.8% agarose gel), a 983 bp fragment should be observed.

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

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