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AuPreP Citations

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AuPreP™ Gold cDNA Synthesis Kit
(For RT-PCR)

Premixed solutions for the synthesis of single-stranded cDNA from RNA for use as a PCR template

Cat. No.: **AUP-CDNA-10GR (10 Rx) / AUP-CDNA-50GR (50 Rx) / AUP-CDNA-100GR (100 Rx)**
Store at: **-20°C**
For long-term storage, Control RNA should be stored at –70˚C.

Product Description: The Polymerase Chain Reaction (PCR) is a powerful technique for rapid amplificationof genes. In addition to amplifying genomic DNA templates, PCR can also be used to amplify cDNA reverse transcribed from RNA to analyze gene expression. Using **AuPreP Gold cDNA synthesis Kit** , RNA is reverse transcribed into single-stranded cDNA. The reverse transcriptase (RT) enzyme synthesizes the new cDNA strand at a site determined by the type of primer used: Oligo(dT) primer, random primer or a sequence-specific primer. The First Strand cDNA can then be used as a template for PCR. **The reaction mix solution supplied, contains buffer, RT with reduced Rnase H activity, dNTP's mix with 99% purity & RNase inhibitor sufficient for 10 Rxs.**

Kit Reagents:		10 Rx	50 Rx	100 Rx
1.	**RT Reaction Mix** contains: Reverse transcriptase with reduced Rnase activity, RNase inhibitor and dNTP's in buffer solution	80µl	400µl	800µl
2.	DTT Solution, 100Mm	20µl	100µl	200µl
3.	Oligo(dT)$_{20}$ Primer, 40µM	10µl	50µl	100µl
4.	Random Hexamer Primer, 40µM	10µl	50µl	100µl
5.	Control RNA Human, Total RNA, 1µg/µl	5µl	25µl	50µl
6.	Primer Mix Human G3PDH amplimers, 10µM each	10µl	50µl	100µl
7.	DEPC-Treated Water	0.3ml	1.5ml	3ml

Additional material required:
- DNA Polymerase for PCR
- Thermal Cycler
- Amplification primers specific to your target cDNA
- Microcentrifuge
- 37°C, 42°C and 70°C water baths or heat blocks

General Considerations:

1. To avoid RNAse contamination and degradation of RNA, wear gloves and use sterile pipette tips.
2. High quality RNA preparation is critical 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 -70°C or below.
3. cDNA Priming:
   AuPreP Gold cDNA synthesis Kit allows the user to choose the desired primer for cDNA synthesis:

   - Gene specific primer: Primer design, conc. and annealing temperature should be evaluated before use.

   - 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.

Procedure:

1. **cDNA Synthesis**
   1.1 Thaw each tube in the **AuPreP Gold cDNA synthesis Kit** and place on ice.
   1.2 Briefly centrifuge all reagents and return to ice.
   1.3 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) or Random Hexamer Primer	1µl (2µM) 1µl (2µM)	Sequence- specific primer may be used at 2 pmole / reaction
DEPC-Treated Water	up to 10µl	

1.4 Mix gently and heat the mixture at +70°C for 5 minutes. Remove tube and place rapidly on ice.
1.5 Add the following components to the reaction tube:

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

Mix gently by flicking the tube.
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.*

1.6 If Random Hexamer Primer is used: incubate at +37°C for 60 minutes
1.7 Other primers: incubate the reaction tubes at +42°C for 60 minutes.
1.8 Heat at +70°C for 15 minutes to stop cDNA synthesis reaction. Centrifuge the mixture briefly to collect the sample at the bottom of the tube.
1.9 At this point the reaction tube, containing the cDNA, may be stored at -20°C or below.
For immediate use, keep tubes on ice.

2. **PCR Amplification**

2.1 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.

2.2 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.

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

3. **Controls**

3.1 **AuPreP Gold cDNA synthesis Kit** 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 the PCR amplification.

3.2 Please use the following PCR protocol for control primers and cDNA template.

3.2.1 PCR reaction:

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

3.2.2 Amplification parameters:

Step	Temperature	Time	
- Initial Denaturation	94°C	2 minutes	
- Denaturation	94°C	45 seconds	30 cycles
- Annealing	60°C	45 seconds	30 cycles
- Extension	72°C	2 minutes	30 cycles
Final extension	72°C	7 minutes	

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