Pro IVT (In-Vitro Transcription) Kit

Cat# no.: U9001

For Research use Only

1.1 Ordering Information

Kit components	U9001-10	U9001-25	U9001-100
10X IVT reaction buffer	20µL	50µL	200µL
Enzyme mix	30µL	75µL	300µL
ATP (100mM)	20µL	50µL	200µL
GTP(100mM)	20µL	50µL	200µL
CTP(100mM)	20µL	50µL	200µL
UTP(100mM)	20µL	50µL	200µL
Nuclease-free water	500µL	1mL	1mL x 2
DNase I	40µL	100µL	400µL
10X DNase buffer	20µL	50µL	200µL
LiCl	200µL	500µL	1mL x 2
Glycogen	10µL	25µL	100µL

1.2 Introduction

In-Vitro Transcription (IVT) is a simple procedure that allows for template-directed synthesis of RNA from DNA template, a fundamental technique in molecular biology. It enables the production of custom-designed RNA sequences for several critical research applications.

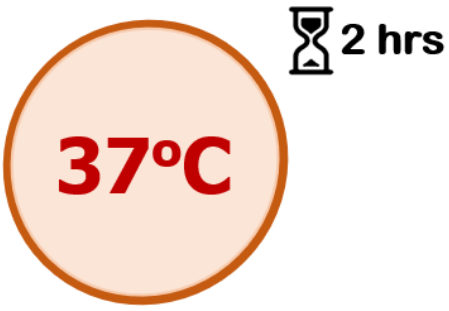
1.3 Description

ProEnz Pro IVT Kit is designed for synthesizing mRNA using a double stranded DNA, containing a T7 promoter, as a template. This kit is used to synthesize high-yield RNA, using approximately 1µg of template. 100µg of mRNA can be obtained in a 20µl reaction volume. The kit provides DNase I for effective digestion of DNA template post-transcription and also provides lithium chloride (LiCl) solution to precipitate and purify synthesized RNA transcript from the reaction components. Glycogen is included as a co-precipitant to improve RNA recovery of difficult to precipitate transcripts.

1.4 Salient features

- In-Vitro transcription
- High yield RNA
- Provided with DNase.

1.5 IVT Conditions



1.5 Storage & handling

Store all the components except buffers at -20 °C and make sure to thaw on ice prior use. Optimal temperature for buffer storage is 2-8°C.

1.6 Protocol

1. Place all the components on ice for thawing 20-30 mins prior.
2. After ensuring that all the components are thawed completely, give a brief spin and place the vials on ice.
3. Place tubes/eppendorfs for the required number of reactions on ice and add the components for 20µl reaction volume as given in Table-1.

Table-1:

Components	Final concentration	Vol. for 20 µL
Nuclease free water	-	Variable
10X IVT Reaction buffer	1X	2 µL
ATP (100mM)	10mM	2 µL
GTP (100mM)	10mM	2 µL
CTP (100 mM)	10mM	2 µL
UTP (100mM)	10mM	2 µL
Template DNA	1ug	Variable
Enzyme mix	-	3ul
		Make up to 20 µL

4. After ensuring that all components have been added to the tube, spin the tubes in a microcentrifuge and ensure no bubbles are visible in the tubes.
5. Place these vials in heat block and perform incubate at 37°C for-2hrs.
6. Start the run and note the end time.
7. The sample should be subjected to DNase treatment before proceeding with purification: for 17µL of sample taken, 2µL of 10X DNase buffer and 1µL of DNase I should be added and this mixture should be incubated at 37°C for 20 mins, followed by 70°C for 10 mins.
8. LiCl purification: For every 10µL of sample, 18.75µL of LiCl solution and 1µL of glycogen should be added. The reaction volume should be made up to 30µL with nucleus-free water. This is followed by incubation at -80°C for 25-30 mins.
9. Centrifuge at 10000rpm for 10-15 mins. Discard supernatant.
10. The pellet should be washed with 1ml of 70% ethanol to remove any salts present. Repeat the wash twice and air-dry the pellet before resuspending it in 30µL nucleus-free water.

Explanation of symbols

REF -Catalogue number

- Expiry

LOT - Lot number/Batch number

-25°C max
-15°C min
- Storage limitations

-Manufacturing: Plot No. 147/D, Phase II, Cherlapally IDA, Telangana, Hyderabad - 500051, India.

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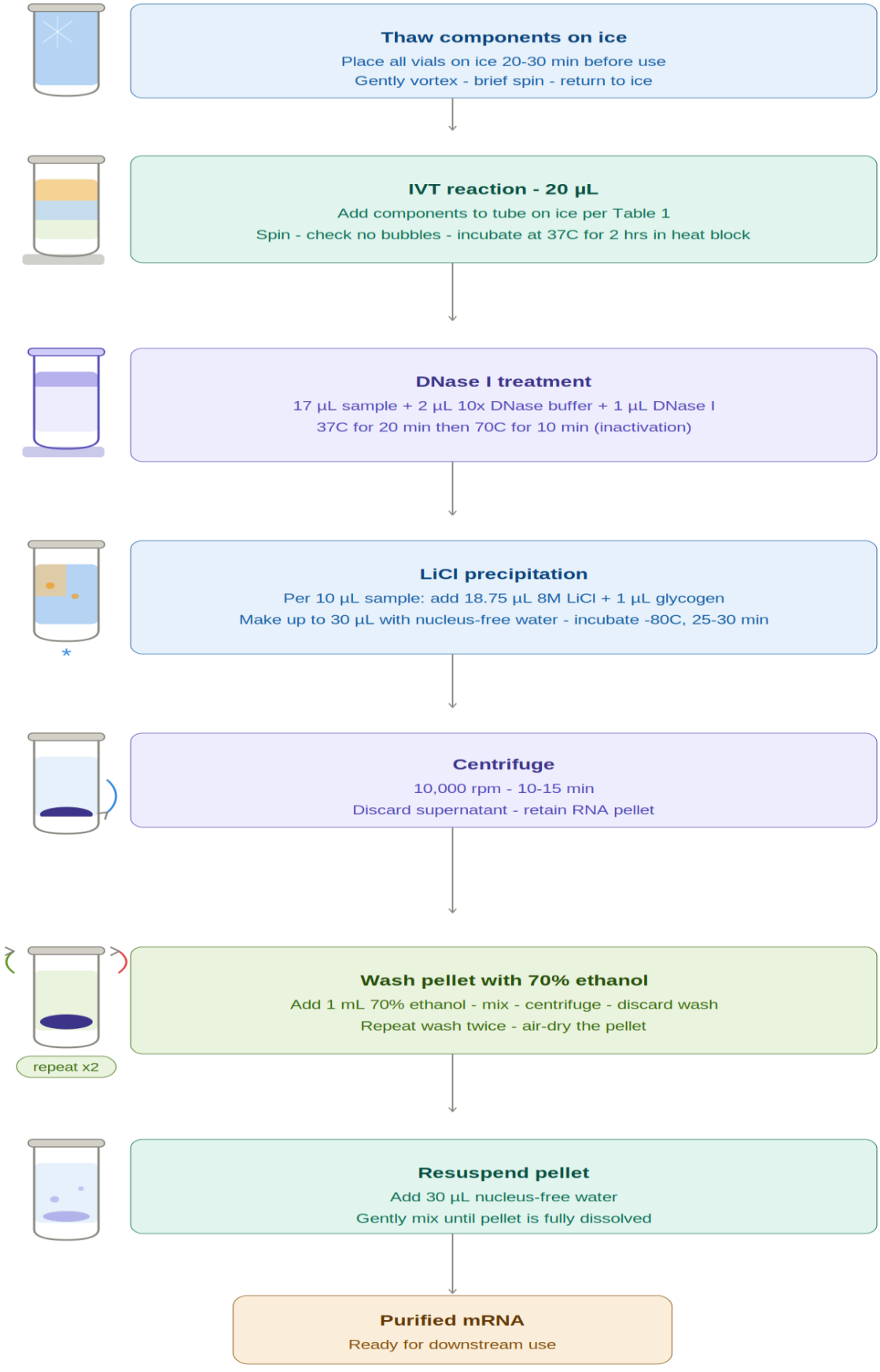
1.7 Schematic representation of workflow



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