RNA production kit

In vitro Transcription Kit

Ready-to-use kit for production of non-capped RNA.

Protocol

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In vitro Transcription kit | Specifications

Package content	**IVT-K25** **Number of reactions : 25**
Shipping conditions	The kit is shipped in dry ice.
Storage conditions	Upon receipt and for long-term use, store the kit at -20°C.
Stability	1 year at -20°C.

For additional information and protocols (optimization, scaling, co-transfection…) tips, troubleshooting or other applications

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Any questions?

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1. Technology

1.1 Description

This kit is **designed for the *in vitro* transcription of your linear plasmid DNA or PCR fragment using the T7 polymerase with a yield of approximately 100 µg of RNA per µg of DNA template**. It is provided with sufficient components for twenty-five reactions (20µl each). Each kit will yield a total of 2.5 mg of RNA. This corresponds to 6.7 nanomoles (25 reactions) of RNA based on the control template provided (1038 nt). The yield will highly depend on each DNA template, sequence, length, and purity.

NOTE:

- Shorter templates typically yield a lower mass and a higher molar yield of RNA product.
- The expected yield of 100µg is achieved with the use of our DNA control template (leading to a 1038 nucleotides IVT product) and non-modified nucleotides. Every modification of the protocol, for example the use of chemically modified nucleotides or Cap analogs can affect the reaction and lead to a diminished yield.

The RNA generated using this kit can be employed in a diverse range of experiments such as *in vitro* translation, cell transfection, isolation of RNA binding proteins, etc..

1.2 Kit composition

The kit is provided with sufficient reagents to perform 25 *in vitro* transcription reactions.

Components	Quantity	Storage
pDNA template Control 1mg/ml (linearized ready-to-use)	5 µl	-20°C
T7 Enzyme mix	50 µl	-20°C
ATP (75mM)	50 µl	-20°C
GTP (75mM)	50 µl	-20°C
CTP (75mM)	50 µl	-20°C
UTP (75mM)	50 µl	-20°C
IVT 10X Buffer	50 µl	-20°C
DNase 1U/µl	25 µl	-20°C
Nuclease free water	1 ml	-20°C
Precipitation solution LiCl 7.5M, EDTA 0.5M pH8	750 µl	-20°C
Gel loading dye 2x 47.5% Formamide, 0.5 mM EDTA, 0.01% SDS, 0.01% SDS, 0.01% Bromophenol Blue, 0.005% Xylene Cyanol)	1ml	-20°C

Material needed not provided with the kit:

- 70% ethanol for RNA washing.
- Nuclease-free buffers (1mM citrate buffer pH 6.4, TE buffer or RNAse-free water) for RNA resuspension.
- Materials or kits for purification of the RNA product.

2. Technology

General Considerations

Before use, thaw all reagents on ice and place the IVT 10X Buffer at room temperature.

NOTE: Spermidine in the buffer can coprecipitate with the DNA at low temperature.

- Purified linear plasmid DNA or purified PCR product can be used for *in vitro* transcription as long as they contain the T7 polymerase promoter. The sequence TAATACGACTCACTATAGGG is the minimum promoter the polymerase need to transcribe the template. The base in yellow correspond to the first base incorporated in the RNA.

- DNA template:

  The DNA template must be linearized downstream of the insert, subsequentially purified and have the T7 RNA polymerase promoter upstream of the sequence of interest. It must also be as clean as possible (free of contaminating protein or RNA). To determine the purity of your DNA, you can measure the A260/A280 and A260/A230 ratios on spectrophotometer. They should be comprised between 1.8 and 2.0 and 2.0 and 2.2 respectively to obtain the greatest IVT yield. PCR templates can also be used as long as they present the T7 promoter upstream of the sequence of interest.

- We suggest to use DNA template at a concentration of 0,5 – 1 mg/ml in RNAse free water or TE buffer (10mM Tris-HCl pH8, 1mM EDTA).

Unit Definition:

- One unit (u) is defined as the amount of enzyme required to incorporate 1 nmol of rATP into the acid-insoluble precipitate within 1 h at 37°C under standard assay conditions.

3. Protocol

The following protocol is designed for the production of 50-100 µg RNA (according to the template size, purity and the use of modified nucleotides) and can be scaled up.

1. Prepare the transcription reaction at room temperature by adding components in the order described in the table below :

Nuclease-free water	To 20 µl
ATP 75 mM	2 µl
GTP 75 mM	2 µl
CTP 75 mM	2 µl
UTP 75 mM	2 µl
IVT 10X Buffer	2 µl
Linear DNA template (or Ctl)	1 µg
Enzyme mix	2 µl

2. Mix by flicking the tube or pipetting up and down.

Microfuge briefly the tube to collect the reaction mix at the bottom of the tube.

3. Incubate 2-4 hours at 37°C.

We recommend 2-hours incubation for templates >1kb and up to 4 hours for templates <1kb. Incubation time can be optimized depending on the size of the template to obtain the best yield.

4. (Optional) Add 1 µl DNase per 20µl-reaction and mix well.

The DNA will be present at a verry low concentration. It may not be a problem according to the application.

a. Incubate for 15 min at 37°C.
b. Place the tubes back on ice.

5. Precipitation of the IVT product.

a. Add 30 µl of RNAse-free water and 30 µl of Precipitation solution to the 20 µL-reaction mix.
b. Mix by flicking the tube or pipetting up and down.
c. Place the tubes over night at -20°C or for a minimum of 2 hours.
d. Centrifuge at 16000*xg* for 15 minutes at 4°C to pellet the RNA.
e. Carefully remove the supernatant without disturbing the pellet.
f. Wash the pellet with 1 ml of 70% ethanol.
g. Centrifuge 5 minutes at 4°C (16000*xg*).
h. Carefully remove the ethanol and air dry the pellet.

4. IVT product quantity and quality determination

4.1 Quantity determination

Quantitation of reaction products by UV light absorbance:
Measure the absorbance of your IVT product at 260nm and determine the concentration according to the following equation:
A260 value x dilution factor x 40 = µg/mL RNA
(For single stranded RNA an absorbance at 260 of 1 correspond to 40 µg/ml).

4.2 Quality determination

1. RNA purity can be determined by calculating the ratio of absorbance at A260/A280. A ratio comprised between 1.8 and 2.0 is generally accepted for high quality RNA.

   NOTE: Any unincorporated nucleotides and/or template DNA in the mixture will affect the reading and estimated RNA concentration.

2. Correct size length of the transcript can be determined by running the IVT product on a denaturing agarose gel electrophoresis. A one or two percent agarose gel containing a nucleic acid stain is sufficient to visualize a wide variety of transcripts (500 nt to 10000 nt).

3. Prepare your sample by diluting 1 µg of RNA in 10 µl of RNAse-free water and mix with 10 µl of Gel Loading Dye 2X. Sample can be Heated at 65°C for 5 min, put on ice before loading to favor proper migration and unwanted secondary structure or double stranded RNA formation. RA will be visualized in the agarose gel using a UV transilluminator. Feel free to run an RNA ladder side to side with the sample to estimate the length of your RNA product.

A capillary electrophoresis instrument can also be used to evaluate the integrity and length of the transcription products.

5. Troubleshooting

5.1 Low yield

Use the control template as reference to determine the yield that is expected to be around 100 µg of RNA.

The amount of RNA synthetized in a 20 µl reaction varies depending on the template used and the incorporation of modified nucleotides.

To enhance the yield of the reaction, the following options can be tested:

1. Increase the quantity of DNA template in the reaction from 1 to 2 µg.
2. Extend the incubation time to 6 hours.

5.2 More than one reaction product – product of the wrong size

1. If the reaction gives a product of shorter size than expected or two RNA products with at least one at the right size: there may be premature termination of the transcription. You can try to lower the incubation temperature to 30°C to solve the problem.

NOTE: Lowering the temperature may result in a lower yield.

2. If the reaction gives a product with a larger size than expected or two RNA products with one at the good size and one at a larger size:

- The linearization of your DNA template may be incomplete. Even a low quantity of circular plasmid will give significant amount of RNA. Try to redigest and purify your DNA template.

- The larger species may be due to a secondary structure of your RNA of interest. Run the reaction product on a denaturing gel and heat the sample at 65°C for 5 minutes before running it on an agarose gel to exclude this possibility.

5.3 Degraded RNA

If your RNA is degraded (smear on agarose gel), some RNAses may be present in your DNA template or introduced during the process. These simple precautions can limit the degradation of your RNA:

1. Wear clean gloves and lab coat.

2. Clean work area and pipette with a surface decontamination agent or with 0,5% SDS and rinse thoroughly with RNAse – free water.

3. Use only dedicated RNAse-free barrier filter pipette tips and RNAse-free water.

4. Keep as much as possible your RNA sample on ice and store the purified RNA at -80°C.

If your DNA template is contaminated with RNAses, additional DNA clean up may be performed. For instance, DNA can be treated by proteinase K (100-200µg/ul) and SDS (0.5%) for 30 min at 50°C and repurify with the method of choice (Phenol-chloroform extraction and ethanol precipitation, or silica-based column).

Additional products for your mRNA production and transfection experiments :

- **Capping Kit** for the *In Vitro* enzymatic capping of RNA
- **Poly(A) tailing Kit** for RNA polyadenylation
- **RmesFect** for mRNA transfection

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