User Manual:

Read all the procedures before using this kit for *in vitro* transciption!!

Material to be supplied by the user

1. Linearized DNA template at 1μg/μl or PCR DNA templates with T7, T3 and SP6 promoters
2. 100% ethanol

***In Vitro* Transcription Experimental Procedure (50μl)**

1. In the SuperTranscrip tube, add the following reagents at room temperature:

Linearized DNA template (1μg/μl)	**2μl**
10X Transcription buffer	**5μl**
RNase inhibitor (5 units/μl)	**1μl**
T7 RNA polymerase Mixture	**8-10 units**
Add nuclease-free water to	**50μl**

2. Mix the solution well and incubate at 37^{o}C for 2 h.
3. Tapping the SuperTranscrip tube several times every 15-20 min to mix the solution (**important!!**)
4. After reaction, add 1μl of DNase I and incubate for 20 min at 37^{o}C.
5. Quick spin the MaxCapping tube and transfer the solution into a new 0.7ml of tube. (at this stage, RNA clean-up kit can also applied for purifying capped mRNA)
6. Add 5μl of 3M NaOAc and 100μl of 100% ethanol.
7. Mix the solution well and centrifuge at >10,000rpm for 20 min to pellet Capped mRNAs.
8. Wash the pellet with 75% ethanol and centrifuge at >10,000rpm for 5 min. Remove the 75% ethanol completely using pipette.
9. Air-dry the pellet for 5min (do not over dry) and add 20-30μl of DEPC-treated nuclease-free water to dissolve RNA pellet.
10. Validate the yield and quality of synthesized capped RNAs by spectrophotometer and agarose gel electrophoresis.

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Troubleshooting:

1. **No transcribed mRNAs**:

- DNA templates are contaminated by RNases: Make sure that DNA template is free of RNase. If ethanol precipitation method is used to purify templates, it is likely that templates may contain RNases. **Suggestion**: Use phenol extraction to remove RNases and all other cellular proteins if ethanol precipitation is used for purifying plasmids.

- DNA templates contain high salts: the most common reason for poor transcription. If DNA templates are prepared by ethanol precipitation after restriction enzyme digestions, DNA templates usually contain certain amount of salts. Since RNA polymerases are very sensitive to salt concentrations, high salt will result in failure of transcription. Also try not to use gel purification method to purify the digest DNA plasmid. Gel purified templates usually give very poor transcripts.
  Suggestion: Use DNA cleaning-up column to remove salt from digested plasmids. Wash the column with washing buffer twice to maximally remove salts.

- Low input amount of DNA templates: Make sure that the mass of DNA template is at least 1-2μg in 50μl of reaction. The 1μg of DNA is not the mass of added plasmids but the fraction of inserts plus RNA polymerase promoter region in the entire plasmid. For example, if the size of the template insert is 1kb and the size of vector backbone is 4kb, totally 5μg of DNA plasmids should be added to make the final mass of "real templates" to 1μg in 50μl of transcription reaction.

2. **Low yield of transcribed capped mRNAs**

- Low input of DNA template: Make of final mass of "DNA templates" at 1μg in 50μl of reaction solution.
- The salt concentration in the reaction solution is too high.
- Too much DNA template was added may also reduce the yields.

3. **Extra bands and smear in gel picture**

- In-completely digestion of DNA plasmids: a large transcript will be made from un-digested templates. (Although gel purification can be used to prepare templates, the yield of capped RNA from gel purified templates is usually low).
- The most common reason for a smear in gel picture is the degradation of synthesized RNAs during electrophoresis. Make sure that the agarose gel and MOPS buffer are RNase-free.

For more questions concerning in vitro transcription and this kit, please visit NeuBiogene.com or send an email to technical_support@neubiogene.com