User Manual:

Read all the procedures before using MaxCapping tubes for *in vitro* capping experiment!!

Experimental Procedure for *in vitro* Capping Transcription (20µl reaction):

1. Take a MaxCapping tube from 4°C refrigerator.
2. Inside the tube, add

DNA templates (1µg/µl)	**1µl**
T7 RNA polymerase mixture (20units)	**1µl**
10X Transcription buffer	**2µl**
10mM cap analog	**1µl**
Add nuclease-free water up to	**20µl**

3. Incubate at 37°C for 90-120 min.
   (**Note**: gently tap the tube several times every 15-20 min to mix the reaction solution)
4. Add 1-2µl of DNase I, and incubate for 20-30min to digest DNA template at 37°C.
5. Add 1/10 volume of 3M sodium acetate and 2 volumes of 100% ethanol.
6. Centrifuge at >10,000rpm for 15-20 min to pellet caped mRNA.
7. Wash the pellet with 70% ethanol wash and centrifuge at >10,000rpm for 5 min.
1. Remove 70% ethanol completely with pipette and air dry pellet for 5 min.
2. Suspend synthesized capped mRNA in 20 µl of DEPC water.
3. Take 2 µl of synthesized capped mRNA to run a gel to verify the quality and use spectrophotometer to estimate the concentration of synthesized capped mRNA.

Troubleshooting:

1. **No transcribed capped 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 because of guanidine.

     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 1µg in 20µl 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 20µl 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**
   - Incompletely digestion of DNA plasmids: a large transcript will be made from un-digested templates.
   - If digested DNA template was purified from gel purification, the yield of capped RNA from gel purified templates is usually low and a smeared small transcript is generally synthesized.
   - 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