

In Vitro Transcription

The transcription protocol is as follows:

- 1 ug template DNA
- 4 ul 5X RIBOMAX buffer
- 2 ul NTPs*
- 1 ul RNAsin
- 1 ul Polymerase
- 1 ul pyrophosphatase
- 20 ul final volume

RIBOMAX Buffer (5X):

- 400 mM HEPES-KOH, pH7.5
- 60 mM MgCl₂
- 10 mM Spermidine
- 200 mM DTT

*NTPs: For cold non-capped transcript, use 25 mM each ATP, CTP, GTP, UTP.

For labeled transcript use 30 mM each ATP, CTP, GTP, and add 1 ul of 1 mM UTP, then label with 100 uCi of ³²-P UTP for a hyb probe, or 10 uCi UTP for a Rnase protection assay probe.

For infectious transcripts, use 30 mM each ATP, CTP, UTP, and 6 mM GTP. Add 6 ul CAP analog at 10 mM, from New England Biolabs.

Incubate at 37 C for 1.5 hours. For capped transcripts add 1 ul of 25 mM GTP after 1 hour.

- Add 1 ul RQ1 RNase-free DNase (Promega)
- Incubate a further 15 minutes.
- Phenol:Chloroform extract.
- Ethanol precipitate with NH₄OAc.

The pyrophosphatase is from Sigma, catalog # I 1891. Resuspend the entire vial in 1 ml of 50% glycerol, and store at -20 as any other enzyme.

For modified SP6 promoters (most of the newer CMV and sat RNA clones) use SP6 RNA polymerase from AMBION only.