mRNA Synthesis

Stocks:

5X Transcription buffer (supplied with enzyme)
0.1M DTT (supplied with enzyme)
ribonucleic acids 100mM each (Roche rNTP set)
10mM rGTP dilution (from Roche rNTP set)
5-methyl G (capG) 20mM (Roche 10904988001)
RNAsin (RNase inhibitor)
RNA Polymerase
Template at 0.5-1 μg/μl
DEPC H_2O

Per Reaction:	**25ul**	**50ul**
1X Transcription Buffer	5ul	10ul
10mM DTT	2.5ul	5ul
2mM each CTP, ATP, UTP	0.5ul (each)	1ul (each)
0.2mM GTP (1:10 stock)	0.5ul	1ul
1mM capG	1.25ul	2.5ul
1-2ug linearized template		
RNasin	1ul	2ul
Sp6 RNA Polymerase	1.25ul	2.5ul
DEPC H_2O	to 25ul	to 50ul
Incubate at 37°C for 2hrs		
DNase treat 30' @ 37°C	1ul	2ul

-run 1ul on 1% check gel (fast run on agarose gel, 5' or less)
-Bring volume to 100μl with DEPC H_2O
-Extract 2X with 100μl Phenol-Chloroform
-Precipitate aqueous phase:
    100ul RNA + 26ul 7.5M NH4OAc + 300ul 100% EtOH
-Spin 30' @ 4°C
-Remove supe and resuspend in 50ul DEPC H_2O
-Precipitate again:
    50ul RNA + 5ul 3M NaOAc + 200ul 100% EtOH
-Spin 30' @ 4°C
-Wash in 70% EtOH, spin 5' @ 4°C
-Resuspend in DEPC H_2O. 30ul for a 25ul reaction is typical
    -Injecting a 1:4 dilution (1ul in 4ul DEPC H_2O) of this RNA (10nl) is about 500pg