In vitro Transcription

Linearize transcription template from your plasmid

Qiagen Clean-up

Add 5 volumes pf buffer PBI to the sample and run it through the column (10K for 30 seconds)
Discard the flow through
Add 750 uL buffer PE and run it through the column (10K for 30 seconds)
Discard the flow through
Dry the column in the microcentrifuge for 1 minute at 13K
Discard the flow through
Add 50 uL of elution buffer, let stand for 60 seconds, and spin at 13K for 1 minute

In vitro transcription

Stock	Final	uL
5x T7 buffer	1X	10
DTT(500mM)	10mM	1
RNaseIn/Out	20U	1
10 mM rA,C,GTPs	.6mM	3
10 mM rUTPs	.6mM	3
1ug template	1ug	6
T7 (Stratagene)	50U	1
H_2O		25

Radioactive Mix

Stock	Final	uL
5x T7 buffer	1X	10
DTT(500mM)	10mM	1
RNaseIn/Out	20U	1
10 mM rA,C,GTPs	.6mM	3
10 mM rUTPs	.1mM	0.5
p32 UTP		10
1ug template	1ug	25
T7 (Stratagene)	50U	1

Incubate for 2hrs @ 37^{o}C
Add 1uL DNase for 15min @ 37^{o}C