Riboprobe Preparation

We use reagents from Promega's Riboprobe System – catalog # depends on polymerase ordered, eg. P1420 (SP6), P1430 (T3), P1440 (T7), P1450 (T3 + T7), P1460 (SP6 + T7))

1. Dry 125 - 250 μCi (10 - 20 μl) ^{35}S-UTP* for each reaction in Speed Vac (use screw top microfuge tubes) (NEG739H Easy Tides, 35S-UTP from NEN)

2. Set up reaction in tube with dried ^{35}S as follows:

Nuclease free water	(9.0 - X) μl
5X buffer	4.0
DTT	2.0
NTP Mix **	3.0
RNAsin	1.0
DNA	X (approx. 1 μg)
Polymerase (T3, T7 or SP6)	1.0
TOTAL	20.0 μl

*10 ul is minimum amount of 35S-UTP, and usually gives good labeling. If probe doesn't label well, try 20 ul.

**NTP mix is 2.5 mM each rATP, rGTP and rCTP (1 ul each)

3. Incubate at 37° C for 75 minutes

4. Add 1 μl RQ1 DNase to each tube- incubate 15 min at 37°C

5. Run over Sephadex G50 column equilibrated with TE

6. Collect fractions by monitoring for first radioactive peak with geiger counter.

8. Precipitate probe by adding 1/10th volume 3 M Na acetate and 2.5 volumes EtOH, in -20°C for 2 hours to overnight. Centrifuge at >12,000g for 10 minutes. Remove supernatant, wash pellet with 70% EtOH, spin 2 minutes, remove supernatant and allow pellet to air dry. Resuspend in appropriate volume (eg. 100 ul) nuclease free H20 with 10mM DTT.

9. Count 1 ul of each collected fraction in scintillation counter.