

Radioactive Marker (for RT-PCR)

1. Mix

- 3 μ l ddH₂O
- 1 μ l DNA (*Msp* I digest) 1 μ g/ μ l
- 1 μ l NEB Buffer 2
- 2 μ l dATP, dGTP, dTTP (2.5 mM)
- 1 μ l Klenow (3' fill, 1/2 u/ μ l)
- 2 μ l α ³²P dCTP

2. Incubate 10 minutes at 37°C

3. Add 1 μ l glycogen, 189 μ l ddH₂O, 200 μ l phenol/chloroform. Spin 2 minutes, max speed, 4°C.
4. Transfer top phase to fresh tube, add 600 μ l 100% EtOH, 100 μ l 5M NH₄OAc. Spin 10 minutes, max speed, 4°C. Look for pellet, and carefully pipette off ethanol and discard.
5. Wash pellet in 200 μ l 70% EtOH. Spin 2 minutes, max speed, 4°C. Carefully pipette off ethanol and allow pellet to air dry.
6. Resuspend pellet in 50 μ l ddH₂O, and add 150 μ l of formamide buffer.
7. For use during the first week, make a 1:10 dilution, or to desired intensity.