PREPARATION OF THE ^{32}P-LABELED OLIGONUCLEOTIDEPROBE

Materials/Reagents:

10X One-Phor-All (OPA) buffer (Amersham)
Sepharose G-50 spin column (Amersham)

Procedure:

Kinasing

1. Mix the following reagents:

oligo DNA	100 ng (~10 pmol)
10X OPA buffer	5 µl
□[^{32}P]-ATP	1 µl (ICN #35020, 7000 Ci/mmol, 5 mCi/30 µl)
T4 DNA kinase (Amersham)	1 µl
dH_2O	to 50□l

2. Incubate at 37˚C for 30 min - 2 hour.
3. Prepare Sepharose G-50 spin column (Amersham): briefly snap off the bottom closure after vortexing well, place in a 1.5 ml tube and spin at 3,000 rpm for 1 min.
4. Place the column in new 1.5 ml tube and apply 50 µl of labeled sample to the top-center of the resin. Spin the column at 3,000 rpm for 2min.
5. The final concentration of the sample is approximately 2 ng (200 fmol)/µl of labeled probe.

Annealing

6. Mix the kinased DNA probe with an excess of the unlabeled complementary strand, for example:

Kinased DNA	20 µl (~40 ng or 4 pmol)
Unlabeled strand	100-200 ng (10-20 pmol)
10X OPA	4 µl
dH_20	20 µl

7. Heat to 95˚C for 5 min and turn off the heat block. Allow the mixture to cool slowly (cover the tube with a lead container). It may take ~3 hour to cool to room temperature. Store at –20˚C.
8. Measure the specific activity of the probe using a scintillation counter. The specific activity should be 1-5x10^5 cpm/µl. The final concentration of double stranded DNA is approximately 2 ng/µl (100 fmol/µl).

Troubleshooting/Critical Parameters

- 1µl of □^{32}P –ATP contains approximately 20 pmol of ^{32}P when the isotope is fresh (more

than enough to label all probes).

- The annealing step is critical for gel-shift. When using a self-annealing probe (for example, 81/81), excess unlabeled strand should be mixed 5 times more. If you label other probes, unlabeled complement probe should be added 10 times amount of labeled DNA.
- If your specific activity is less than 100,000 cpm/pmol (10,000 cpm/µl), you should do kinase reaction again.
- Use 1-10 fmol of probe for gel-shift.
- You can monitor the amount of probe forming double strands by running on 15% polyacrylamide gel in 1XTBE. Compare between before and after annealing step. If band was up-shifted completely, the probe is OK.

81/81 (self-anneal) : 5'-CTAGAAGCTTCTAGAAGCTTCTAG-3'
human Hsp70 HSE
Forward: 5'-GAGGCGAAACCCCTGGAATATTCCCGACCTGGC-3'
Reverse: 5'-GCCAGGTCGGGAATATTCCAGGGGTTTCGCCTC-3'

Reference:
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