NICKIT p.s.o., Probe Size Optimization Kit

Cat. No. MB-1905

Vector's **PHOTOPROBE® Biotin** and **FastTag™ systems** both label DNA probes intended for *in situ* hybridization (e.g. Daniel *et. al*, 1998; Lee *et. al*, 1993; Murashov and Wolgemuth, 1996). However, these methods label without nicking the nucleic acid. As a consequence, the length of the labeled probe may be larger than optimal for *in situ* hybridization applications. Several groups (e.g. Koji *et. al,* 1988; Moench *et. al*, 1985) have shown that a probe size of <400 bases results in optimal signal with low background, probably due to better tissue penetration.

Traditionally, large DNA probes have been reduced to the appropriate size by partial digestion with *E. coli* deoxyribonuclease I (DNase I). However, to avoid over- or under-digestion, continual titration of the DNase I is required. In order to simplify probe digestion, Vector Laboratories has developed a procedure for reducing the size of DNA probes to their optimal length, without the problem of over-digestion, using a specially prepared T4 endonuclease V which cleaves the phosphodiester bond at pyrimidine dimers formed by U.V. irradiation.

The procedure involves a brief U.V. irradiation of probe DNA using a transilluminator or hand-held U.V. lamp followed by digestion with a specially prepared T4 endonuclease V. Digestion of probes should be done prior to labeling.

NICKIT p.s.o. kit components:

- 15 µl stabilized T4 endonuclease V (For long term storage, store at -20 °C.)
- 150 µl T4 endonuclease V reaction buffer (Store at 4 °C to -20 °C.)

The following reagents are required in addition to the T4 endonuclease V digestion kit reagents.

- transilluminator ($\lambda \leq 310$ nm) or hand-held U.V. lamp ($\lambda \approx 254$ nm)
- laboratory film (e.g. PARAFILM®†) or plastic wrap
- phenol (equilibrated in buffer)
- 10 M ammonium acetate* **or** 1.5 M sodium acetate, pH 5.2 **or** 1 M NaCl
- 1 M $MgCl_2$*
- 95% ethanol*

** These reagents are available in an accessory reagent kit, Cat. No. SPK-1902.*

† PARAFILM is a registered trademark of American Can Company

Protocol:

Warning! Exposure to U.V. light is hazardous! Please utilize proper safety precautions.

1. Place ≤20 µg of DNA in 40 µl of H_2O or TE (10 mM Tris, 1 mM EDTA, pH 8) on a sheet of laboratory film or plastic wrap.
   See Note A.

2. Place the laboratory film on a transilluminator ($\lambda \leq 310$ nm) and irradiate for 20 minutes.
   See Note B.

   Alternatively, place a hand-held U.V. lamp ($\lambda \approx 254$ nm) approximately 0.5 to 1.0 cm above the DNA sample and irradiate for 40 minutes. Due to variation in the fluence of different lamps, some optimization of irradiation time may be required.
   See Note C.

3. Transfer the irradiated DNA to a microcentrifuge tube. Adjust the volume to ~31 µl with distilled water. (The volume will have been reduced somewhat by evaporation.) Add 8 µl of reaction buffer and 1 µl of the T4 endonuclease V solution. Mix.

4. Incubate at 37 °C for 2 hours.

5. Add 40 µl of phenol equilibrated in buffer (see Sambrook *et. al*, 1989, for preparation protocol) and vortex vigorously.

6. Separate the phases by centrifugation at 13,000 x g for several seconds.

7. Transfer the aqueous (upper) phase to another microcentrifuge tube.

8. Precipitate the DNA by adding the following components and mixing:

   9.5 µl H_2O
   13 µl 10 M ammonium acetate
   2.5 µl 1 M $MgCl_2$
   163 µl 95% ethanol (cold)

   Incubate at -20 °C for 20 minutes. Pellet the DNA by centrifugation at 13,000 x g for 20 to 30 minutes.
   See Note D.

9. Resuspend the DNA pellet in 10 to 40 µl of TE. Proceed to FastTag™ or PHOTOPROBE® Biotin labeling procedure.
   See Note E.

Notes:

Note A. DNA samples should contain less than 10 mM salt because high salt concentrations reduce the processivity of T4 endonuclease V.

Note B. Removal of the clear low wavelength filter which is attached to the surface of many U.V. transilluminators is recommended to increase the efficiency of pyrimidine dimer formation.

Note C. An irradiation "stand" can be made by laying a pencil on either side of the DNA sample and resting the hand-held lamp face-down on top.

Note D. Ethanol precipitation is imperative because traces of phenol inhibit labeling using aryl azide derivatives such as FastTag™ or PHOTOPROBE® Biotin. Either 13 µl of 1.5 M sodium acetate (pH 5.2) or 13 µl of 1 M NaCl may be substituted for the ammonium acetate in step 8.

Note E. The size of the digested DNA can be verified by comparison of a sample of the digestion product with a DNA molecular weight standard following agarose gel electrophoresis. Nicked, double strands migrate slower than single strands. Therefore, samples should be heat denatured and quick-chilled immediately prior to electrophoresis.

References:

Daniel SG, Westling ME, Moss MS and Kanagy BD. 1998. FastTag™ nucleic acid labeling system: A versatile method for incorporating haptens, fluorochromes and affinity ligands into DNA, RNA, and oligonucleotides. *BioTechniques* 24:484-89.

Koji T, Moriuchi T and Nakane PK. 1988. Improved tissue preparation for *in situ* localization of specific mRNA using non-radioactive DNA probes: Effects of protease digestion and probe size on signal detection in frozen and paraffin sections of rat pituitary glands. *Acta Histochem. Cytochem.* 21(2):187-200.

Lee BL, Unabia G and Childs G. 1993. Expression of follistatin mRNA by somatotropes and mammotropes early in the rat estrous cycle. *J Histochem Cytochem* 41(7):955-60.

Moench TR, Gendelman HE, Clements JE, Narayan O and Griffin DE. 1985. Efficiency of *in situ* hybridization as a function of probe size and fixation technique. *J. Virol. Methods* 11:119-130.

Murashov AK and Wolgemuth DJ. 1996. Sense and antisense transcripts of the developmentally regulated murine hsp70.2 gene are expressed in distinct and only partially overlapping areas in the adult brain. *Brain Res Mol Brain Res* 37(1-2):85-95.

Sambrook J, Fritsch EF, and Maniatis T. 1989. Molecular Cloning: A Laboratory Manual. 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.