

## **EABiotech Ltd.**

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### **<sup>3</sup>H-Methyltransferase Assay.**

A sample of EcoKI DNA methyltransferase which had been covalently labelled with <sup>3</sup>H-methyl-S-adenosyl methionine prior to separation of the labelled protein subunits via SDS-polyacrylamide gel electrophoresis and transfer of the sample to a PVDF membrane (Immobilon P, Millipore) by electroblotting, was prepared for Enhanced Autoradiography by melting the EA-Wax into it.

An identical sample was prepared for fluorography by soaking the SDS polyacrylamide gel in an enhancer solution (Amplify, Amersham) prior to drying the gel on to paper.

Both samples were exposed at -70 °C to preflashed x-ray film (Cronex4, Dupont) for 3 days. As can be seen the sample prepared for Enhanced Autoradiography (lanes 6, 7, 8, and 9) produced a signal which is approximately 3 fold greater signal than that prepared with the Amplify (lanes 1, 2, 3, and 4).

That the wax can be removed from the PVDF blot by two minutes gentle shaking in toluene, allowing the total protein to be visualised by Coomassie Blue staining (lane 11).

Lanes 5 and 10 are the same samples as shown in lanes 4 and 9, but with an exposure of only 16 hours. The arrows at the side of lane 11 show the position of the molecular weight markers.

Advantages of Enhanced Autoradiography in this experiment:

1. An image with better resolution is obtained.
2. The sample is easily recovered for further procedures.

Alternative treatments such as amino acid sequencing or protein detection using antibodies should now be feasible.

It should now be possible to apply this method to the detection of tritiated ligonucleotides, DNA, RNA, protein-DNA complexes and many other systems which can be placed on a solid support such as a filter membrane or chromatogram.

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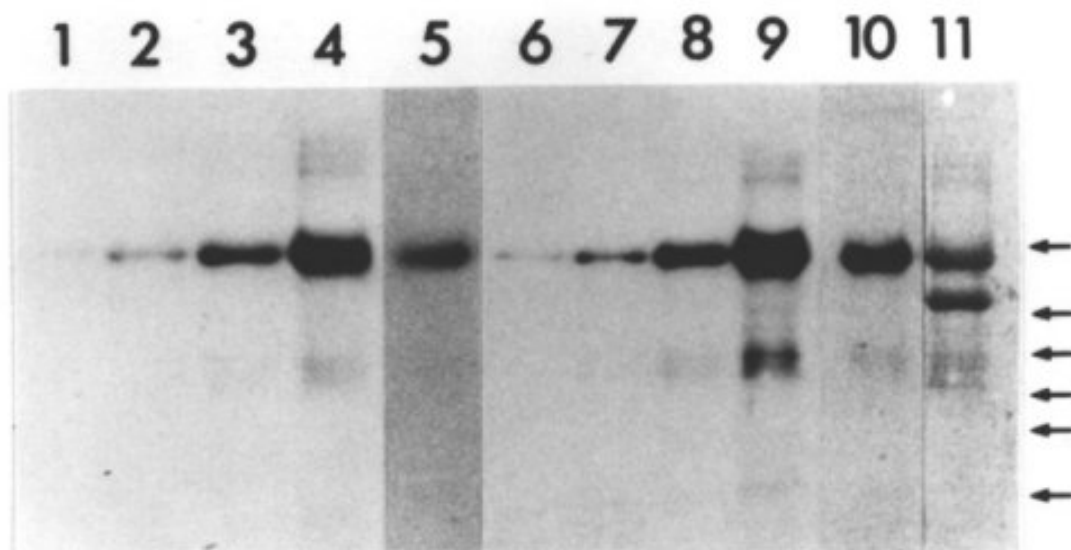


Figure 1. *EcoKI* DNA Methyltransferase labelled with  $^3\text{H}$ -methyl-S-adenosyl methionine.

Lanes 1 to 4 processed using Amersham Amplify. Three day exposure.

Lanes 6 to 9 processed using Enhanced Autoradiography. Three day exposure.

Lane 11 is lane 9 wash with toluene and stained with Coomassie Blue to demonstrate sample recovery. The arrows show the molecular weight markers.

Lanes 5 and 10 are lanes 4 and 9, but with an exposure of only 16 hours.