

EABiotech Ltd.

Unit 4, Strathclyde Business Centre, 416 Hamilton Road, Flemington. G72 7XR Scotland

Telephone: 0141 646 2820 Facsimile: 0141 646 2830

e-mail: info@eabiotech.com website: www.eabiotech.com

Detection of tritiated(³H) labelled compounds after Thin Layer Chromatography (TLC).

Samples of tritiated(³H) labelled CTP, UTP, GDP, and TTP were separated using thin layer chromatography(TLC). All compounds were spotted onto the TLC plate as a 2m l sample of stock solution of 1m M, and the specific activities of the stock were as follows:

CTP: 1.7kBq; UTP: 2.7kBq; GDP:2.5kBq; TTP:6.2kBq.

Standard autoradiography was used in an attempt to visualise the spatial distribution of the various tritiated(³H) labelled compounds. The TLC plate was exposed to preflashed X-ray film , in a cassette, which was placed in a -700C freezer . After 8 weeks the film was developed and was found to be blank.

The same TLC plate was then prepared for Enhanced Autoradiography. The EA-Wax was melted into the TLC plate at a temperature of 550C for a period of 5 minutes. After cooling, the plate was again placed in a cassette with preflashed X-ray film, and placed in a -700C freezer. The film was developed after a period of 7 days. The resultant images are shown in Figure 1.

The advantages of Enhanced Autoradiography in this experiment are:

1. The superior detection efficiency of Enhanced Autoradiography over autoradiography is obvious, by visual inspection. The tritiated(³H) labelled compounds are all clearly visible.
2. An adequate image could well be available in a shorter time.
3. Autoradiography produced no result after 8 weeks. It could be more than double this period of time before a usable image was produced.

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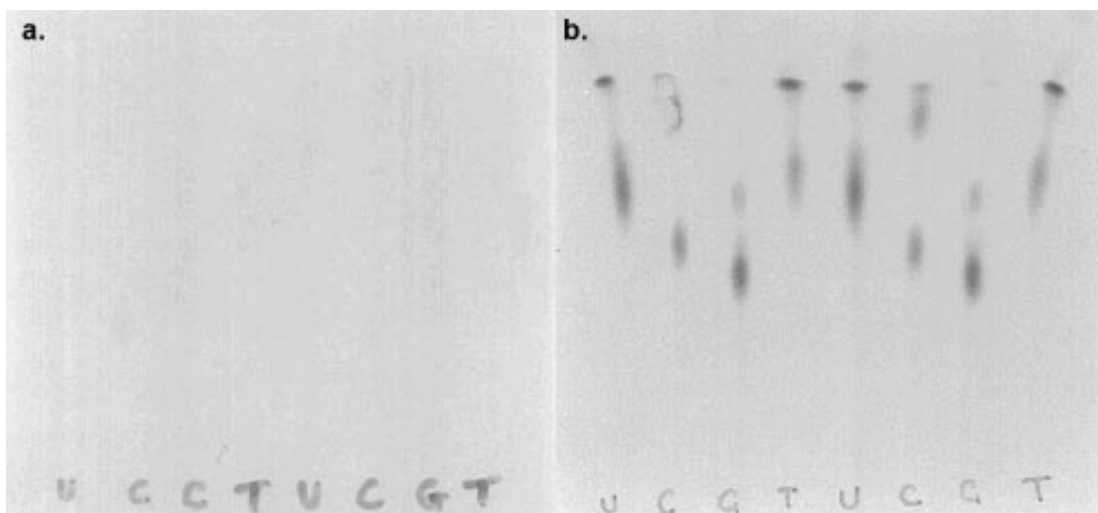


Figure 1. **Detection of tritiated(^3H) labelled compounds after Thin Layer Chromatography.** a) TLC plate image after 8 weeks using conventional autoradiography, b) The same TLC plate as in figure 1 but after 7 days using Enhanced Autoradiography