AlkPhos Direct / ECF

Introduction

The AlkPhos Direct™ Labelling System and Enhanced Chemifluorescence, ECF™, are techniques suitable for applications such as Southern and Northern blots. AlkPhos Direct Labelling reaction involves labelling nucleic acid with a specially developed thermostable alkaline phosphatase enzyme. The enzyme, which is in the labelled probe, converts the ECF substrate to a fluorescent precipitate that is detected by ImageMaster™ VDS-CL. A light emission maximum at 560 nm is captured when the blots are illuminated with UV light (maximum excitation at 430 nm).

The use of this non-radioactive method allows safe handling and permits reliable, highly sensitive detection of target DNA. Using the ECF detection system together with ImageMaster VDS-CL enables quantification of target DNA.

Images of Southern blots acquired by ImageMaster VDS-CL camera and ImageMaster 1D Elite software are shown below. After a 24 hour incubation with substrate, ImageMaster 1D Elite software can yield reliable DNA quantification down to 250 fg of a single copy gene per band.

Results

Digested and diluted DNA was separated in a 1% agarose electrophoresis gel and blotted to a Hybond™ N^+ membrane. An AlkPhos Direct Labelled N-ras probe was hybridized to the membrane. Stringency washes and addition of substrate were followed by a 24 hour incubation with substrate. The blots were subsequently exposed to UV light for 10 seconds to generate a signal. The light emitted was detected and an image was captured by ImageMaster VDS-CL.

Figure 1a shows Southern blots after 24 hour incubation and 10 seconds exposure. The 4th dilution corresponds to 250 fg target DNA. (For high target applications, an acceptable result can be obtained after 1 hour.)

Figure 1b is a graph showing complete linearity in quantification, i.e. the relation between loaded amounts of DNA and fluorescent light output is linear.

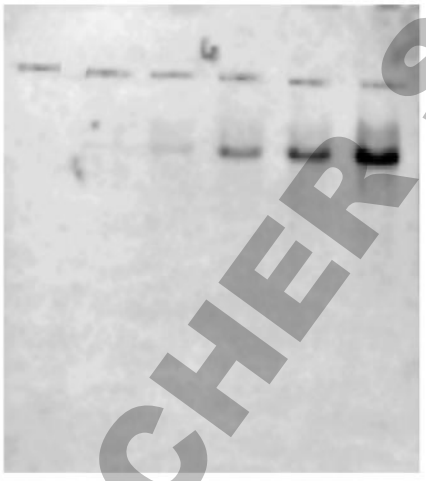

Fig. 1a. A Southern blot with a dilution of Hind III digested Human Genomic DNA on Hybond N^+. The loadings were: Lane 1, 4 µg; Lane 2, 2 µg; Lane 3, 1 µg; Lane 4, 0.5 µg; Lane 5, 0.250 µg; Lane 6, 0.125 µg. The blot was exposured for 10 seconds following a 24 hour incubation with substrate. The band in lane 4 is visualized and corresponds to a 250 fg loading.

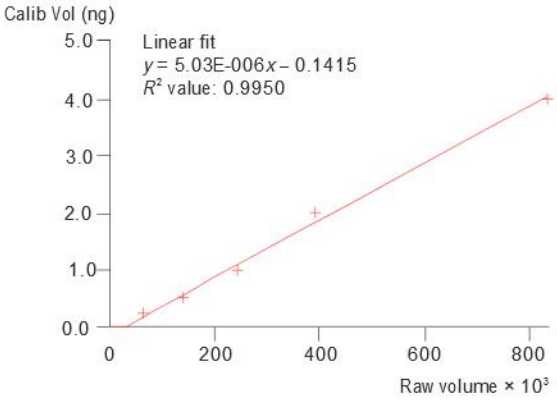

Fig. 1b. A graph from Southern blot showing the relationship between the amount of target DNA and the relative fluorescent light output determined from a quantitative analysis of the membrane image. The linear equation used to fit the data was $y = 5.03E\text{-}006x - 0.1415$; R^2 value: 0.9950.

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Conclusion

AlkPhos Direct Labelling and Enhanced Chemifluorescence, ECF, are suitable techniques for Southern blots, especially for low target applications and when quantification is essential.

Materials and methods

Human placental genomic DNA was digested with Hind III and diluted in 1× Blue Juice. Loadings of 4, 2, 1, 0.5, 0.25, 0,125 µg per lane were applied to 1% agarose SepRate™DNA Agarose, 1×TAE, and separated using GNA 200, 80 V for 2 hours. After separation, the DNA was transferred to a Hybond N^+ membrane through capillary blotting in 10×SSC. The DNA was cross-linked to the membrane by UV cross-linking in UVC 500 UV Cross-Linker at energy setting 700. The insert, N-ras (1.5 Kb), was labelled using AlkPhos Direct. The labelling procedure, pre-hybridization, hybridization, stringency washes and signal generation were done according to the manufacturer's instructions.

Imaging using ImageMaster VDS-CL

The blots were analysed with ImageMaster VDS-CL using the UV light tray.

The ImageMaster VDS-CL parameters for capturing an image of the blots were:

Iris = 4, Filter = UV high, Gain = 200,
Exposure time = 10 seconds, and 12 Bits Tiff. The UV upper lamp was used.

The image was imported into ImageMaster 1D Elite software for further analysis, such as quantification. The amounts of target DNA in the different lanes were estimated and found to be completely linear (Fig. 1b). Even very low amounts of target DNA were estimated correctly.

References

Ordering information

18-1130-55	ImageMaster VDS-CL
80-6350-37	ImageMaster 1D Elite Software
18-1132-28	ImageMaster TotalLab
RPN3680	AlkPhos Direct Labelling Module
RPN 3685	ECF Detection Module
RPN 2020B	Hybond N^+ Membrane
80-6222-31	UVC 500 UV Cross-Linker

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