

SCIENCE IMAGING SYSTEMS

Application Note No.18

Northern Blot Detection Using CSPD[®] Chemiluminescent Reagent

LAS-1000 / LAS-1000plus

Foreword

The introduction of the LAS series of cooled CCD cameras made it possible to detect even weak chemiluminescence with good sensitivity. Moreover, chemiluminescent reagents have become easier to use owing to various improvements, including higher emission intensity, longer emission lifetime and the availability of reagent kits. The combination of these advances has allowed trace amounts of DNA, RNA, proteins and other substances to become easily detected by chemiluminescence.

A particularly attractive feature of chemiluminescent detection is that it can be conducted in an ordinary laboratory. Many researchers are trying to adopt new chemiluminescence detection system in place of existing RI-based test systems.

Dr. Akiko Fukushima, the principle author of this Application Note, uses the chemiluminescence method for detection of Northern blotting in her work in the Department of Molecular Nutrition, at Kagawa Nutrition University, particularly for the detection of genes expressed in the small intestine. In the following, she gives a detailed description of the test conditions and protocol used.

Contents

1. Protocol
2. Test results
3. References

Summary

- Northern blot detection is possible with CSPD[®] chemiluminescent reagent.
- All the procedures can be carried out in an ordinary laboratory because no RI are used.
- Sensitivity of this method is comparable to RI methods.

1 Protocol

■ Reagents

- ISOGEN (Nippon Gene Co., Ltd.)
- Oligotex™-dT30<Super> (Takara Shuzo Co., Ltd.)
- Agarose ME (FMC BioProducts)
- Nylon membranes, positively charged (Roche Diagnostic systems)
- DIG RNA Labeling Kit (SP6/T7) (Roche Diagnostic systems)
- DIG Luminescent Detection Kit (Roche Diagnostic systems)

Takara Shuzo Co., Ltd.

<http://www.takara.co.jp/>

Roche Diagnostic systems

<http://www.roche.com/diagnostics/>

■ Protocol

RNA preparation

Scrape mucous membrane from small intestine onto glass slide and prepare total RNA^{*1} using ISOGEN.

^{*1} When bands cannot be detected using total RNA, purify to poly(A)⁺RNA with Oligotex™-dT30<Super> or the like.

Membrane preparation

- (1) Electrophorese 0.8% agarose gel^{*2} containing formaldehyde using a Mupid-3 mini-gel electrophoresis apparatus (Cosmo Bio Co., Ltd.)
- (2) Add 4.0μl of 5x MOPS buffer solution, 3.6μl of formaldehyde and 10μl of formamide to 2.4μl of RNA solution^{*3} and react for 15min at 60°C.
- (3) After the reaction, add 2.0μl of dye solution and electrophorese at 50V.
- (4) Transfer RNA separated by electrophoresis to positively charged nylon membrane by blotting with 20x SSC.
- (5) Maintain membrane at 120°C for 30min to fix RNA.

^{*2} To prepare, dissolve 0.8g of agarose in 50ml distilled water in an autoclave, next add 12ml of distilled water, 20ml of 5x MOPS buffer solution and 18ml of formaldehyde, and then pour into a gel tray.

^{*3} 20μg if total RNA, about 2.0μg if poly(A)⁺RNA should be contained in this 2.4μl RNA solution.

Cosmo Bio Co., Ltd.

<http://www.globe.or.jp/cosmobio/>

Hybridization

- (1) Prepare RNA probe using DIG RNA Labeling Kit (SP6/T7) in accordance with enclosed protocol.
- (2) Add 5.0ml of hybridization solution^{*4} to membrane (11 x 6cm) and conduct hybridization at 65°C for at least 3hr.
- (3) Discard the solution and add to 1.0ml^{*5} hybridization solution containing DIG-labeled RNA probe and react overnight (about 15 hours) at 65°C.
- (4) Wash reacted membrane with 2x SSC, 0.1% SDS at room temperature for 10min. Further wash twice with 0.2x SSC and 0.1% SDS at 65°C for 20min each.

^{*4} 5x SSC, 10x Denhardt's solution, 10mM sodium phosphate buffer pH6.5, 0.5% SDS, 50% formamide, 0.1mg/ml salmon milt-derived DNA.

^{*5} Amount of hybridization solution varies with size of membrane.

Experimental Tip

In the hybridization step (3), the concentration of the DIG-labeled RNA probe is not clearly mentioned. In this case, 2.5µl of the probe was added to 1.0ml of the hybridization solution, based on the fact that the amount of DIG-labeled probe was about 1/5 that of the DIG-labeled RNA control.

Detection

- (1) Conduct detection with DIG Luminescent Detection Kit in accordance with manufacturer's directions.
- (2) Dilute CSPD® 100 fold with detection buffer*⁶.
- (3) Seal membrane (11 x 6cm) and add 0.5ml*⁷ of the CSPD® solution in hybridization bag.
- (4) Bring sealed membrane to steady state by incubating at 37°C for 15min.
- (5) Measure chemiluminescence with LAS-1000plus imaging system (Fuji Photo Film Co., Ltd.).

*⁶ 10mM Tris-HCl, pH9.5, 0.1M NaCl

*⁷ Amount of CSPD® solution varies with size of membrane.

Experimental Tip

This protocol prepares the RNA probe and conducts northern hybridization using a DIG RNA Labeling Kit (SP6/T7). Although sensitivity is slightly lower, substantially the same results can also be obtained by labeling with a DIG DNA Labeling Kit (Roche Diagnostic Systems), conducting hybridization at 50°C and washing at 55°C.

Experimental Tip

In our laboratory, we use DIG system northern hybridization for detection of various genes expressed in the small intestine (calcium-binding protein (calbindin D9k), lactase, sucrase, vitamin D receptor, β-actin). While sensitivity differs between genes depending on amount of gene expression and RNA properties, we obtained sensitivity comparable to that using the RI method. Genes that cannot be detected by the RI method unless purified to poly(A)⁺RNA are also difficult to detect by the DIG system unless similarly purified.

2 Test results

We detected calcium-binding protein of rat small intestine by LAS-1000plus with CSPD® chemiluminescent reagent (Fig.2-1). The results of image analysis is shown in Fig.2-2, in which the horizontal axis is scaled for total RNA and the vertical axis for the value determined. As shown in Fig.2-2, linear results were obtained.

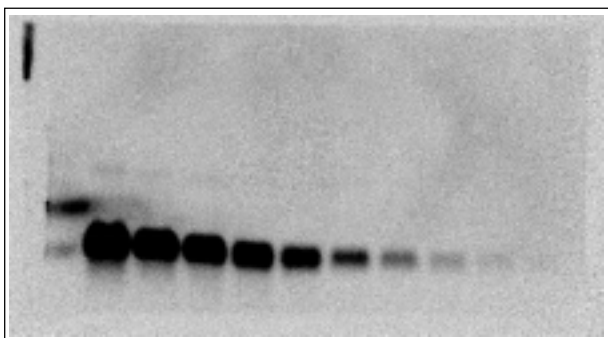


Fig.2-1

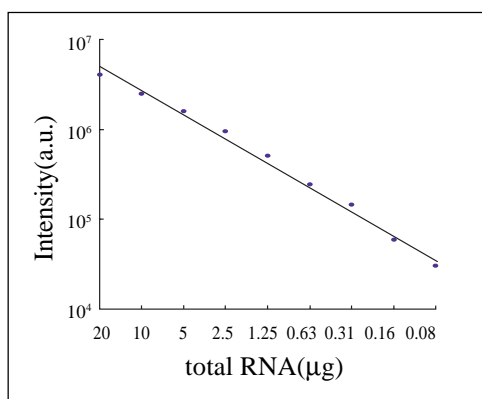


Fig.2-2

Fig.2-1 Image of calcium-binding protein (calbindin D9k) from rat small intestine obtained by Northern blotting

Amount applied: From left, 20μg, 10μg, 5μg, 2.5μg, 1.25μg, 625ng, 312ng, 156ng, 78ng

Substrate: CSPD®

< LAS-1000plus parameters >

Binning : NO

Exposure Time : 1min

Position : 1

Fig.2-2 Results of Image Analysis

3 References

- 1) Kohri K., Nomura S., Kitamura Y., Nagata T., Yoshioka K., Iguchi M., Yamate T., Umekawa T., Suzuki Y., Sinohara H. and Kurita T., Structure and expression of the mRNA encoding urinary stone protein (osteopontin), J. Biol. Chem., 268, 15180-15184 (1993).
- 2) The DIG system user's guide for filter hybridization, Roche Diagnostic systems.

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