



# The DIG System – a High Sensitive Substitute of Radioactivity in Northern Blot Analysis

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## Introduction

The Northern blot technique is frequently employed for the characterization of gene expression, RNAs and for gene function analysis. Using radioactively-labeled probes is still a common technique in Northern blot analysis. The main disadvantages of this technique are the possible health hazard, inconvenience during handling and the short half-life of the probe. Here we demonstrate that the digoxigenin (DIG) system is an easy and very sensitive alternative in Northern blot analysis. DIG-labeled probes are stable for at least one year and guarantee short detection times. By analyzing the transcription pattern of the mRNA of the human DEAD box protein p68 RNA helicase [1] in different tissue types it is shown that DIG labeling is comparable to radioactive labeling. Furthermore an optimized stripping protocol opens up new possibilities for stripping and reprobing of Northern blots.

## Materials and Methods

A human Multiple Tissue Northern blot (MTN-Blot, CLONTECH), with poly(A)<sup>+</sup> RNA from various human tissues was employed.

Radioactive labeling and detection was performed according to standard protocols. A p68-specific cDNA fragment, representing the 1300 bases at the 5'-end of the human p68 cDNA [2], cloned into an appropriate transcription vector, was labeled by in-vitro transcription with <sup>32</sup>P-ATP. Hybridization was performed with DIG Easy Hyb Buffer (1 x 10<sup>6</sup> cpm <sup>32</sup>P-labeled RNA probe/ml) overnight. The blot was washed under high-stringency conditions and analyzed by autoradiography.

Non-radioactive labeling and detection was performed according to the DIG System Application Manual for Filter Hybridization [3] or the pack insert of the DIG Northern Starter Kit. The same p68-specific cDNA fragment as for the radioactive approach and a p68 intron 11 specific *Stu I* - *Mun I* 1017-bp fragment was DIG labeled

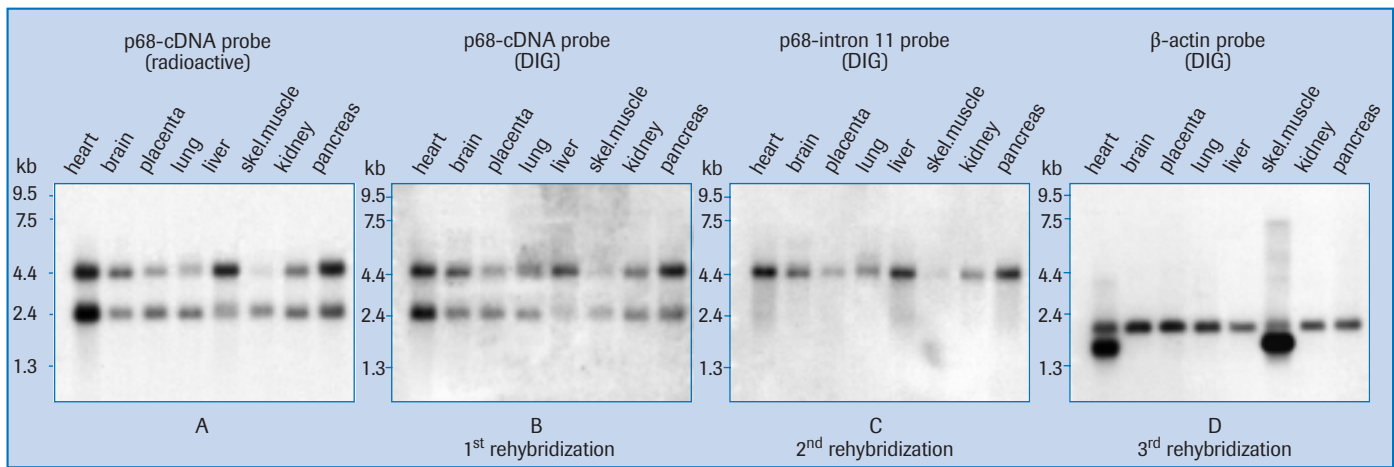
by in-vitro transcription using the DIG Northern Starter Kit. To quantify the RNA loaded in each lane, a DIG-labeled human  $\beta$ -actin probe was used. Hybridization was performed with DIG Easy Hyb Buffer (100 ng DIG-labeled RNA probe/ml) for 6 hours at 68 °C. The blot was then washed under high-stringency conditions and detection was performed using the DIG Wash and Block Buffer Set and CDP-*Star* chemiluminescent substrate, making sure that the membrane never dried during hybridization and detection. The blots were exposed to the Lumi-Imager F1 Workstation for 5 minutes.

For stripping of RNA probe a stripping solution (always freshly prepared) containing 50% formamide (de-ionized), 5% SDS, 50 mM Tris/HCl, pH 7.5 was used. The blot was rinsed briefly in DEPC- or DMDC-treated water, then incubated in 12 ml stripping solution twice for 30–60 minutes at 80 °C in a sealed bag in a preheated shaking water bath. Finally the blot was briefly rinsed in 2xSSC. The blot was stripped soon after detection and stored wet in 2xSSC at 4 °C up to the next detection, taking care that the membrane never dried out.

## Results and Applications

In this study the distribution of the p68 mRNA in various human tissues by using a Multiple Tissue Northern (MTN) blot was analyzed. Initially, the blot was hybridized with a <sup>32</sup>P-labeled p68 cDNA-specific anti-sense RNA probe. Two classes of p68 transcripts of approximately 2.4 and 4.4 kb in size were detected in the poly(A)<sup>+</sup> RNA from each tissue analyzed (Figure 1A), similar to the results reported previously for rodent tissues [4]. To compare the efficiency of DIG-labeled probes, the same transcript was then DIG labeled and incubated with the stripped blot. As shown in Figure 1B, identical signals were detected after a 5-minutes exposure to Lumi-Imager F1 Workstation using CDP-*Star* chemiluminescent substrate.

Size comparison by gel electrophoresis of in-vitro transcripts of full length p68 cDNA identified the 2.4-kb



**Figure 1: Northern blot analysis of RNA of eight different human tissues.** Each lane was loaded with poly(A)<sup>+</sup> RNA from different human tissues as indicated. The blot was first incubated with a <sup>32</sup>P-labeled p68 anti-sense RNA probe representing a p68 cDNA fragment (A; one day exposure to Fuji RX film). The membrane was stripped and reprobred with the same probe labeled with DIG (B). The blot was stripped again and reprobred with a p68 intron 11 derived anti-sense RNA probe (C). Finally the blot was stripped and reprobred with an anti-sense human β-actin RNA probe (D). The DIG-labeled probes were detected using CDP-Star chemiluminescent substrate and the blot was exposed to Lumi-Imager F1 Workstation for 5 minutes

product as the mature p68 mRNA (data not shown). Hence, the 4.4-kb RNA was hypothesized to represent alternatively spliced variant(s). To analyze this, the blot was stripped and incubated with a probe specific for intron 11, the largest of the human p68 gene introns [5]. The position of human intron 11 is conserved in the p68 related gene in *Saccharomyces cerevisiae* and its presence had previously been shown to be involved in post-transcriptional regulation of this gene [6]. Using the intron 11-specific probe, only the 4.4-kb RNA was detected (Figure 1C). This clearly identified p68 splice variants as abundant poly(A)<sup>+</sup> RNA in the steady-state pool of human tissues. The 4.4-kb RNA seems not to be expressed. Its abundance rather hints to a regulatory process involved in maturation of p68 primary transcripts.

To compare the RNA load in each lane, the blot was stripped again and rehybridized with a DIG-labeled human β-actin probe (Figure 1D).

The stripping procedure used in these experiments overcomes the problems seen before in stripping RNA probes. The DIG-labeled probes were completely removed (compare Figure 1B, 1C, 1D) and no loss of target was observed (compare Figures 1B, 1C). Moreover, using the described protocol it was possible to strip and reprobe a Northern blot up to 20 times (data not shown).

## Summary

We have successfully used the DIG system in Northern blot analysis with regard to sensitivity and the reprobred of the blots. Its use provides considerable advantages

over the commonly used radioactive procedure. These are short detection times, safety aspects and the enhanced stability of the DIG-labeled probes.

## References

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<http://biochem.roche.com/dig>



Product	Pack Size	Cat. No.
<b>DIG Northern Starter Kit</b>	1 kit (10 labeling reactions and detection of 10 x 10 cm <sup>2</sup> membrane)	2 039 672
<b>Lumi-Imager F1 Workstation</b>	240 Volt 120 Volt	2 015 170 2 012 847
<b>DIG Easy Hyb Granules</b>	granules for 6 x 100 ml	1 796 895
<b>Anti-Digoxigenin-AP, Fab fragments</b>	150 U (200 µl)	1 093 274
<b>CDP-Star, chemiluminescent substrate (ready-to-use)</b>	2 x 50 ml	2 041 677
<b>Actin RNA Probe, DIG-labeled</b>	2 µg	1 498 045
<b>Digoxigenin-11-UTP</b>	250 nmol (25 µl)	1 209 256
<b>DIG Wash and Block Buffer Set</b>	1 set (30 blots)	1 585 762
<b>Nylon Membranes, positively charged</b>	10 sheets (20 x 30 cm) 20 sheets (10 x 15 cm) 1 roll (0,3 x 3 m)	1 209 272 1 209 299 1 417 240
<b>Hybridization Bags</b>	50 bags	1 666 649
<b>mRNA Isolation Kit</b>	1 kit	1 741 985
<b>Buffers in a Box, premixed SSC Buffer, 20x</b>	4 l	1 666 681