

# G:BOX iChemi XR & XT for imaging ribonuclease protection assays

## A powerful method for detecting low abundance mRNA

### Introduction

Gene Expression and protein activity within a cell are of great interest to researchers. Changes in cellular mRNA levels are of particular interest as these changes directly correlate in their corresponding protein levels, there are however exceptions to the rule. Many scientists study gene expression by monitoring the response of mRNA molecules to genetic manipulations this is often accomplished by using a number of different techniques including Northern blotting, RT-PCR and nuclease protection assays.

### Advantages of RPA

RPA has several advantages compared to other techniques used such as, Northern blots which depend on binding RNA to a solid support, as RPA assays can more readily detect even low abundance mRNA in a solution of total RNA.

RPA assays also provide greater sensitivity when using small amounts of total RNA, and using a non-radioactive chemiluminescent approach. Syngene has developed the G:BOX iChemi XR & XT automated chemiluminescent image analysers, these systems are ideal for non-radioactive RPA work as they provide hands free automated series image capture, while their cooled CCD cameras allow long integration times with low background noise.

### Materials and Methods

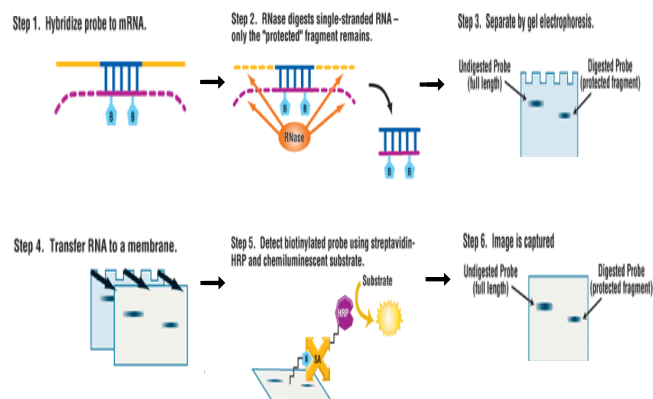
#### Generating and hybridizing non-radioactively labelled RNA

*In vitro* transcript RNA probes are simultaneously synthesised and labelled with a non-radioactive marker such as biotin (Figure 1).

#### Detecting hybridisation

To visualise hybridisation of the biotinylated RNA probe, a detection system such as the BrightStar™ BioDetect™ Kit (Ambion, Huntingdon, UK) is used.

The blot is placed inside the G:BOX iChemi XR/XT's light tight darkroom and images are captured using the GeneSnap image acquisition software. G:BOX iChemi's Extended Dynamic Range (EDR) can then be applied combining up to 65 thousand captured light exposures. Using EDR the detection of faint chemiluminescent bands can be achieved. The optimised image can be analysed automatically using the GeneTools software.



**Figure 1- Schematic of RPA protocol**

Probes (in 3-10 fold molar excess to the total RNA) and total RNA are hybridised (Step 1). The hybridised probes are then treated with RNase A and T1 to degrade any single-stranded RNA. Labelled probe and complementary RNA complex is protected from RNase digestion and is run on denaturing polyacrylamide gel (Steps 2 and 3). The polyacrylamide gel is transferred to a nylon membrane. The membrane is treated with chemiluminescence before being visualised (Steps 4, 5 and 6). Image adapted from Piercenet.com

### Results

Using a G:BOX iChemi XR/XT with a chemiluminescent RPA, researchers have reported being able to produce RPA blot images with a high signal to noise ratio. This has enabled them to identify mRNA specific to their proteins of interest from as little as 5 - 50 ng mRNA, in a solution of 5-20 µg total RNA.

### Conclusions

Syngene's G:BOX iChemi XR/XT provide a sensitive method for effectively detecting small amounts of mRNA in a non-radioactive RPA. Since the software allows series image capture and EDR researchers can save hands on time, while still generating one perfectly exposed RPA blot image. Analysing this image with GeneTools software enables the generation of quantitative data, a task that is difficult to perform using X-ray film.

***Syngene reserves the right to amend or change specifications without prior notice. This Application Note supersedes all earlier versions.***

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