

DIG System Sensitivity and Specificity

High specificity and sensitivity are the reason, researchers worldwide choose the DIG System to detect nucleic acids using filter and *in situ* hybridization. Compared to radioactivity less target material is needed for robust results because sensitivity is high.

The DIG labeling moiety is the steroid hapten digoxigenin, isolated from digitalis plants (*Digitalis purpurea* and *Digitalis lanata*), the natural source of digoxigenin. Anti-DIG antibodies are highly specific and do not nonspecifically bind nucleic acids, preventing background smears that can occur when using streptavidin/biotin.

The DIG System is ideal for nucleic hybridization analysis. Immobilized nucleic acids are hybridized with a DIG-labeled probe. Subsequent detection is performed using high affinity antidigoxigenin antibodies, coupled either to alkaline phosphatase (AP), horseradish peroxidase (POD), and fluorescein or rhodamine for colorimetric, chemiluminescent or fluorescent detection (see Figure 1).

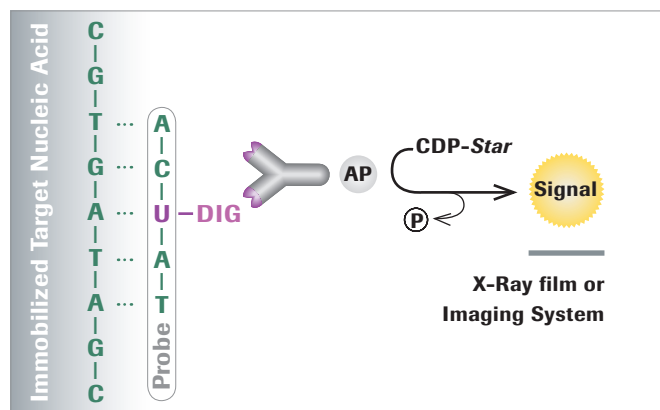


Figure 1: Example detecting DIG-labeled nucleic acids using chemiluminescence substrates.

For life science research only.
Not for use in diagnostic procedures.

Target Concentrations Using DIG – Labeled Probes

It is easy to use proven Roche protocols.

For Northern blot hybridization, use DIG-labeled probes to target 10 µg to 30 µg of total RNA. With the DIG-system, resulting chemiluminescent signals are so intense even brief exposures can produce strong signals with a background showing the lanes.

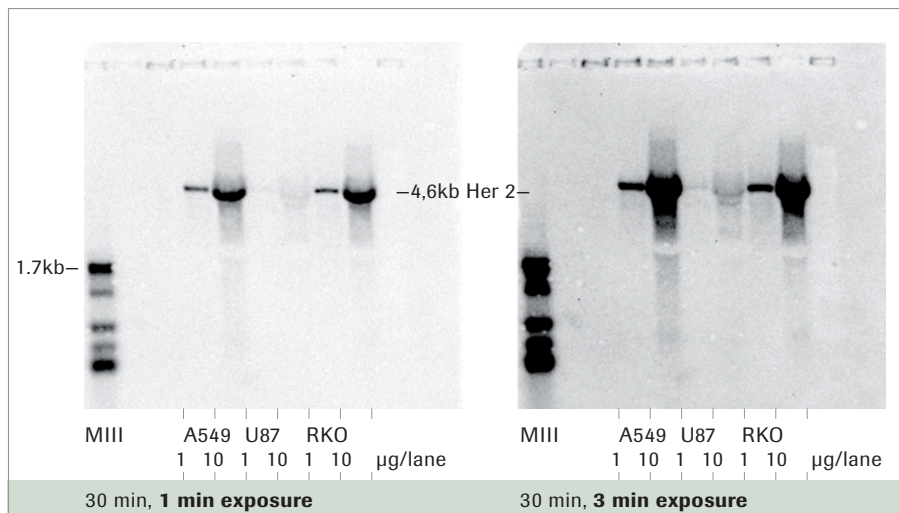


Figure 2. DIG-labeled Her2 antisense RNA probe hybridization to total RNA isolated from A549, U87, and RKO cell lines; 10 µg and 1 µg target total RNA were used for northern blotting. Her2 mRNA was detected as a clear band with the expected size of 4.6 kb. The above shows 1 min and 3 min exposure times after 30 min of incubation using the Roche chemiluminescent reagent CDP-Star. Lanes with 10 µg total RNA appear overloaded, so that nonspecific hybridization is evident, and longer exposures are not possible.

For best results, load 1 µg and 500 ng total RNA when using DIG-labeled RNA probes. Use 5 µg total RNA for each lane when DIG-labeled DNA probes are used. These RNA amounts are sufficient to detect even low abundant mRNAs. As shown in Figure 2, the weak expression of Her2 in U87 cells is readily detected in 1 µg total RNA using a 3 min exposure. Signals using 10 µg total RNA are too intense after even very brief exposures.

Use Proven Roche DIG Protocols

Achieve specific and sensitive hybridization using the DIG System. As the examples below show, it is important to use the appropriate hybridization buffer to minimize nonspecific hybridization.

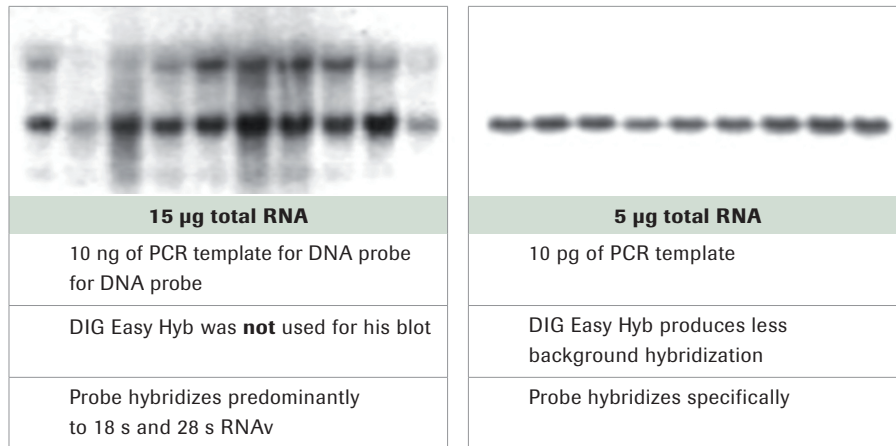


Figure 3. Too high a template concentration when generating the PCR-labeled DNA probe and using a “home brew” hybridization buffer (left) can produce nonspecific hybridization signals. For best results, follow the recommended protocol and use the DIG Easy Hyb Hybridization Buffer (right). Data provided courtesy of Hanlin Gao, University of Columbus, Ohio, USA.

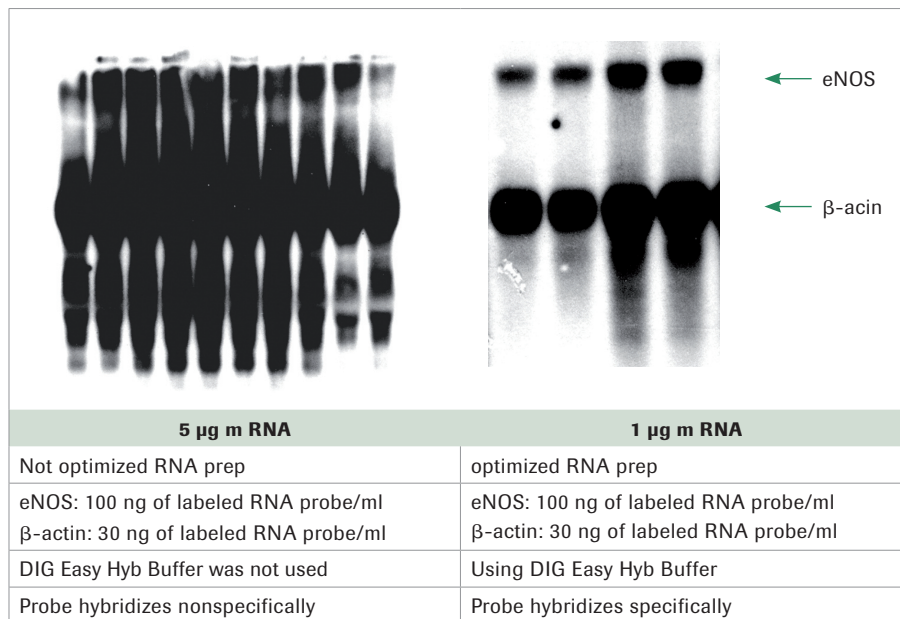


Figure 4. High target concentration and a “home brew” hybridization buffer (left) can produce nonspecific hybridization signals. For many applications, it is sufficient to use total RNA for filter hybridization, and not necessary to blot mRNA. For best results quickly, use Roche’s *DIG Easy Hyb* Hybridization Buffer (see results in Figures 3 & 4 above). Data provided courtesy of G. Egink, University Köln, Germany.

Summary

DIG-labeled probes are sensitive and ideal for use in specific filter and *in situ* hybridization. Compared to other labeling and detection methods, DIG's high sensitivity permits using lower amounts of target material (*i.e.*, total RNA).

This conserves precious sample material when performing filter hybridization. For best results quickly, follow the easy-to-use protocols and in the package inserts.

Further Reading

For best results, use the information provided in the package insert of Roche's **DIG Northern Starter Kit**.

Additional information and results using the DIG System can be found in:

Roche Cancer Research Application Note No. 10, Weil et al., June 2011: Cancer Research Workflow: Analysis of Nutlin-Induced Changes in mRNA Synthesis and Protein Expression using the xCELLigence System, qRT-PCR, Northern Blots, and Western Blots.

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Published by

Roche Diagnostics GmbH
Sandhofer Straße 116
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