Dig System for Starters

Content

Powerful and Versatile DIG System

Roche Applied Science was one of the first companies to offer a nonradioactive technology, eliminating hazardous radioactive isotopes. After 2 decades, the **DIG System** remains one of the most popular nonradioactive technologies to label and detect nucleic acids for multiple applications, such as filter or in situ hybridization.

Advantages of Nonradioactive Labeling and Detection:

- **Eliminate health** risks to end user.
- **Obtain higher sensitivity** compared to radioactivity.
- **Achieve results quicker** compared to isotopic procedures.
- Store probes for **more than one year**.
- Employ **easy-to-use** protocols.
- **Buy everything from one supplier** – all products for all applications.

Digoxigenin (DIG) is a steroid hapten derived from Digitalis plants. It can be used for labeling of nucleic acids and proteins for hybridization, and subsequent color or luminescence detection. It can be incorporated into DNA, RNA, or oligonucleotides via labeled uridine nucleotides, to which digoxigenin is linked via a spacer arm containing carbon atoms.

Note: Compared to biotin, which occurs endogenously in almost every tissue and cell, DIG shows no nonspecific side reactions when working with crude DNA preparations or in tissues and cells. Additionally, streptavidin, which is primarily used for the detection of biotin, tends to stick nonspecifically to all solid supports, such as microplates and membranes, resulting in high backgrounds.

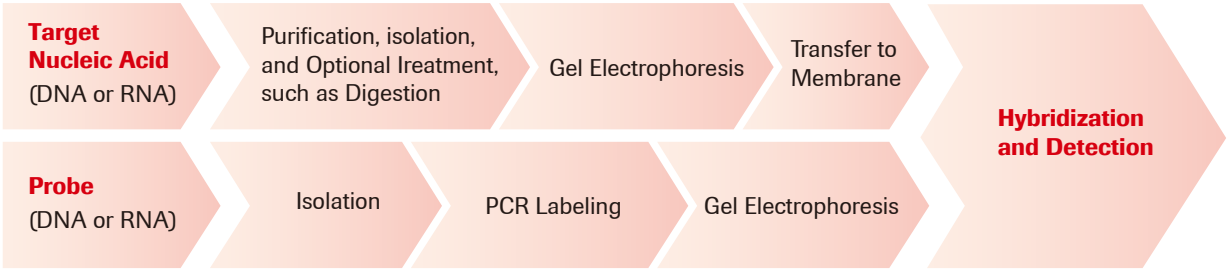

Figure 1: Overview of the DIG blot hybridization workflow using PCR.

2. Labeling Nucleic Acids using the DIG System

Labeling Nucleic Acids using the DIG System

DIG-labeled nucleotides may be incorporated, at a defined density, into nucleic acid probes by DNA polymerases, as well as RNA polymerases (SP6, T3, or T7 RNA), and terminal transferase. The most straightforward way to add the DIG label is PCR for DNA labeling (exemption probes longer than 10 kb) and in vitro transcription for RNA (easily synthesized by direct in vitro transcription of DNA templates, which are either cloned into the respective in vitro transcription vectors or amplified by PCR, adding the appropriate RNA polymerase priming sites). Nevertheless, several other sophisticated options are available.

2.1 DIG DNA Labeling by PCR

PCR labeling is the preferred method for preparing DIG-labeled hybridization probes or labeled amplicons. Only small amounts of template are necessary, even as little as 60-80 bp. There is less demand on purification quality of the template DNA and only a few optimization steps are required, compared to other methods. The process provides high yields of labeled probe and allows for sensitive detection of amplicons (approximately 100-1000 times more sensitive than agarose gels stained with ethidium bromide).

TIP

For DIG-labeling of hybridization probes, use the PCR DIG Probe Synthesis Kit. The PCR DIG Probe synthesis kit contains a 1:2 ratio of DIG-dUTP versus dTTP. Therefore, it provides the ideal sensitivity when making hybridization probes for applications, such as Southern and northern blotting.

For DIG-labeling of amplicons, use the PCR DIG Labeling Mix or the PCR DIG Labeling Mix^{PLUS}. The DIG PCR Labeling Mix contains a 1:20 ratio of DIG-dUTP to dTTP. Therefore, it provides the ideal sensitivity when labeling amplicons for applications, such as ELISA.

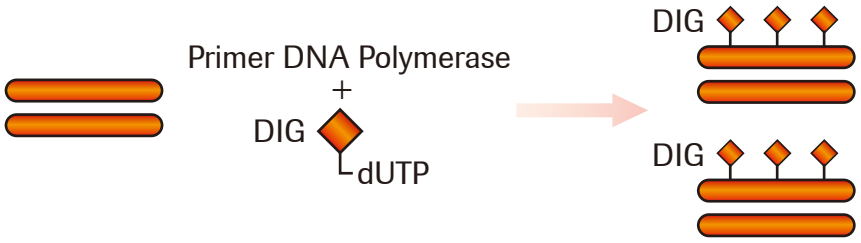

Figure 2 : DNA Labeling by PCR. Thermostable polymerase incorporates DIG-dUTP as it amplifies a specific region of the template DNA. The result is a highly labeled, specific, and sensitive hybridization probe.

Note: The polymerase chain reaction (PCR) allows the amplification of minute amounts of DNA to levels above 1 µg. The only prerequisite is that some sequence information of the target sequence is needed in order to synthesize the appropriate primers.

2.2 Labeling RNA Probes using the DIG System

In vitro transcription of RNA labeled with DIG using RNA polymerases (SP6, T3, or T7 RNA) can be accomplished with the DIG RNA Labeling Kit (SP6/T7). RNA Polymerases, such as T7 or T3 were used for *in vitro* transcription, and incorporation of DIG-dUTP was done using the DIG RNA Labeling Mix. For those new to northern blotting, the DIG Northern Starter Kit is the ideal, ready-to-use convenient solution. Everything you need in a complete kit: RNA labeling with digoxigenin and SP6/T7/T3 RNA Polymerases, and CDP-Star for chemiluminescent detection.

3. Critical Hints for PCR Labeling

Critical Hints for PCR Labeling

PCR is a powerful tool for the amplification of nucleic acids. However, optimization is necessary for proper performance, such as avoiding the formation of nonspecific products. The same is true for DIG incorporation via PCR.

TIP

PCR is a straightforward and highly recommended method for DIG labeling. The PCR DIG Probe Synthesis Kit, which contains the Expand High Fidelity Enzyme Blend, is ideal as it requires less optimization. You can efficiently use GC-rich templates without optimization of the MgCl2 concentration. Most labeling reactions will work with the standard 1.5 mM MgCl2 concentration.

Note: The PCR DIG Labeling Mix is especially designed for the sensitive analysis of PCR reactions by direct detection of PCR products using DIG. The PCR DIG Labeling Mix can replace the unlabeled nucleotide mix in PCR. It is not suited for the preparation of hybridization probes, as it contains only a ratio of 1:20 DIG dUTP:dTTP. For the direct detection of PCR products, separate the PCR products on an agarose gel, transfer to a membrane, and perform the detection reaction. For such applications, the lower concentration of 1:20 DIG dUTP:dTTP is sufficient.

3.1 PCR Conditions

Optimize PCR amplification parameters (cycling conditions, template concentration, primer sequence, and primer concentration) for each template and primer set in the absence of DIG-dUTP before attempting incorporation of DIG.

3.2 Template

For best results, use cloned inserts as the template. As primary extension products are co-amplified linearly, use isolated inserts for labeling to prevent undesired cross-hybridization of vector DNA. Genomic DNA can be more difficult to use.

Note: During the initial cycles, so called "primary extension products" are generated because the polymerase can run past the priming sites. These long products may contain vector sequences, or in case of complex genomic DNA, undesired repetitive element sequences or even unrelated products from secondary priming sites. The long products that run past the priming site are co-amplified linearly, and can eventually cause undesired signals during hybridization.

TIP

Template concentration is critical to successful production of specific probes. Use 10-100 pg plasmid DNA. Higher template concentrations result in higher portions of labeled vector sequences (primary extension products) and background. Use 1 - 50 ng of complex genomic DNA. Generation of probes by direct amplification from human genomic DNA can be complicated by secondary priming sites and primary extension products that run past the priming sites.

3.3 Special Considerations for Long Templates

For templates <1 kb, the standard ratio of DIG-dUTP:dTTP provided by the PCR DIG Probe Synthesis Kit results in high labeling efficiency at optimal amplification. Longer templates have a risk of not being efficiently amplified using the standard conditions.

TIP

PCR probes longer than 1 kb are preferably labeled with a DIG-dUTP:dTTP ratio of 1:6. The 1:6 ratio can be easily prepared by mixing 1 volume Of DIG Labeling Mix from the PCR DIG Probe Synthesis Kit (vial 2; ratio1:2) with 1 volume of the dNTP Stock Solution (vial 4), not containing DIG.

3. Critical Hints for PCR Labeling

3.3 Special Considerations for Long Templates

Generally, ratios between 1:3 and 1:10 can be used. However, the 1:2 and 1:6 ratios were chosen to provide the highest sensitivity in complex applications. We do not recommend 1: 20 mixes.

Additionally, longer templates (>3 kb) need specific conditions for the amplification. To amplify longer templates, use the Expand Long Range dNTPack.

TIP

Labeling of PCR probes longer than 3 kb may be optimized using the Expand Long Range dNTPack. Optimization of the DIG-dUTP:dTTP ratio is required for these templates.

3.4 Purification

High-purity nucleic acids are a priority for any labeling reaction. Purification of labeled probes from unincorporated DIG-dUTP prior to hybridization is not necessary. If a probe causes background problems (for example, if the template DNA was isolated using a home-brew assay), the High Pure PCR Product Purification Kit offers a convenient solution.

3.5 Evaluation of the Labeling Efficiency

Labeling efficiency of PCR-labeled probes can easily be performed via gel electrophoresis on an agarose gel using ethidium bromide staining. DIG-labeled PCR products show a significant increase in molecular weight due to the digoxigenin label. By comparing with unlabeled PCR product on an agarose gel, the efficiency of labeling can be evaluated. For PCR-labeled probes, it is therefore not necessary to perform a dilution series and a direct detection.

TIP

Typically, 2 µl of DIG-labeled PCR product per milliliter of hybridization solution is used for the hybridization. If the PCR product signal is very faint, up to 4 µl can be used; if the PCR product signal is very strong, as little as 0.5 µl will be sufficient. Therefore, one PCR reaction of 50 µl total volume (after removal of 5 µl for agarose electrophoresis) generates enough probe for approximately 650 cm2 membrane, using 3.5 ml hybridization solution per 100 cm^2 membrane.

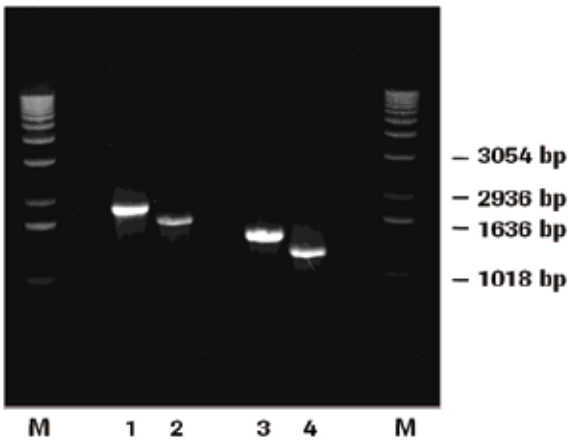

Figure 3 Two different DIG-labeled, single-copy probes (lanes 1 and 3) compared to the respective unlabeled amplification products (lanes 2 and 4). The DIG-labeled PCR products show a significant increase in molecular weight due to the high labeling density with DIG. This can be easily detected in comparison to the unlabeled PCR product on an agarose gel.

3. Critical Hints for PCR Labeling

3.6 Storage of DIG labeled Probes

Due to high labeling efficiency and amplification rate, PCR-labeled hybridization probes are ideally suited for long-term use. In contrast to radioactively labeled hybridization probes, the stability of DIG-labeled hybridization probes exceeds 18 months when stored at -20°C.

Ordering Information

Product Name	Cat. No.	Pack Size
PCR DIG Probe Synthesis Kit	11 636 090 910	1 kit for 25 reactions of 50 µl final reaction volume (one reaction can produce enough labeled probe to analyze 650 cm^2 of blotting membrane)
PCR DIG Labeling Mix	11 585 550 910	500 µl (2 x 250 µl) for 2 x 25 reactions of 100 µl final reaction volume (final concentration 200 µM)
PCR DIG Labeling Mix^{PLUS}	11 835 289 910	2 x 250 µl for 2 x 50 reactions of 50 µl final reaction volume
DIG RNA Labeling Kit (SP6/T7)	11 175 025 910	1 kit for 2× 10 labeling reactions
DIG RNA Labeling Mix	11 277 073 910	40 ul for 20 labeling reactions
T7 RNA Polymerase	10 881 767 001	1,000 U
T7 RNA Polymerase	10 881 775 001	5,000 U
T3 RNA Polymerase	11 031 163 001	1,000 U
T3 RNA Polymerase	11 031 171 001	5,000 U
DIG Northern Starter Kit	12 039 672 910	1 kit for 10 labeling reactions and detection of 10 blots of 10 x 10 cm^2
Expand Long Range dNTPack	04 829 034 001	175 U for up to 50 reactions of 50 µl final volume, each containing 3.5 U enzyme blend
Expand Long Range dNTPack	04 829 042 001	700 U for up to 200 reactions of 50 µl final volume, each containing 3.5 U enzyme blend
Expand Long Range dNTPack	04 829 069 001	3,500 U (5 x 700 U) for up to 1000 reactions of 50 µl final volume, each containing 3.5 U enzyme blend
High Pure PCR Product Purification Kit	11 732 668 001	1 kit for up to 50 purifications
High Pure PCR Product Purification Kit	11 732 676 001	1 kit for up to 250 purifications

License Disclaimer

Expand Long Range dNTPack:
48: Use of this product is covered by one or more of the following US patents and corresponding patent claims outside the US: 5,789,224 (exp. 4/8/2014), 5,618,711 (exp. 4/8/2014), 6,127,155 (exp. 10/3/2017), and claims outside the US corresponding to US Patent No. 4,889,818 (expired in US). The purchase of this product includes a limited, non-transferable immunity from suit under the foregoing patent claims for using only this amount of product for the purchaser's own internal research. No right under any other patent claim (such as the patented 5' Nuclease Process claims in US Patent Nos. 5,210,015 (exp. 8/6/2010), no right to perform any patented method, and no right to perform commercial services of any kind, including without limitation reporting the results of purchaser's activities for a fee or other commercial consideration, is conveyed expressly, by implication, or by estoppel. This product is for research use only. Diagnostic uses under Roche patents require a separate license from Roche. Further information on purchasing licenses may be obtained by contacting the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA.

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