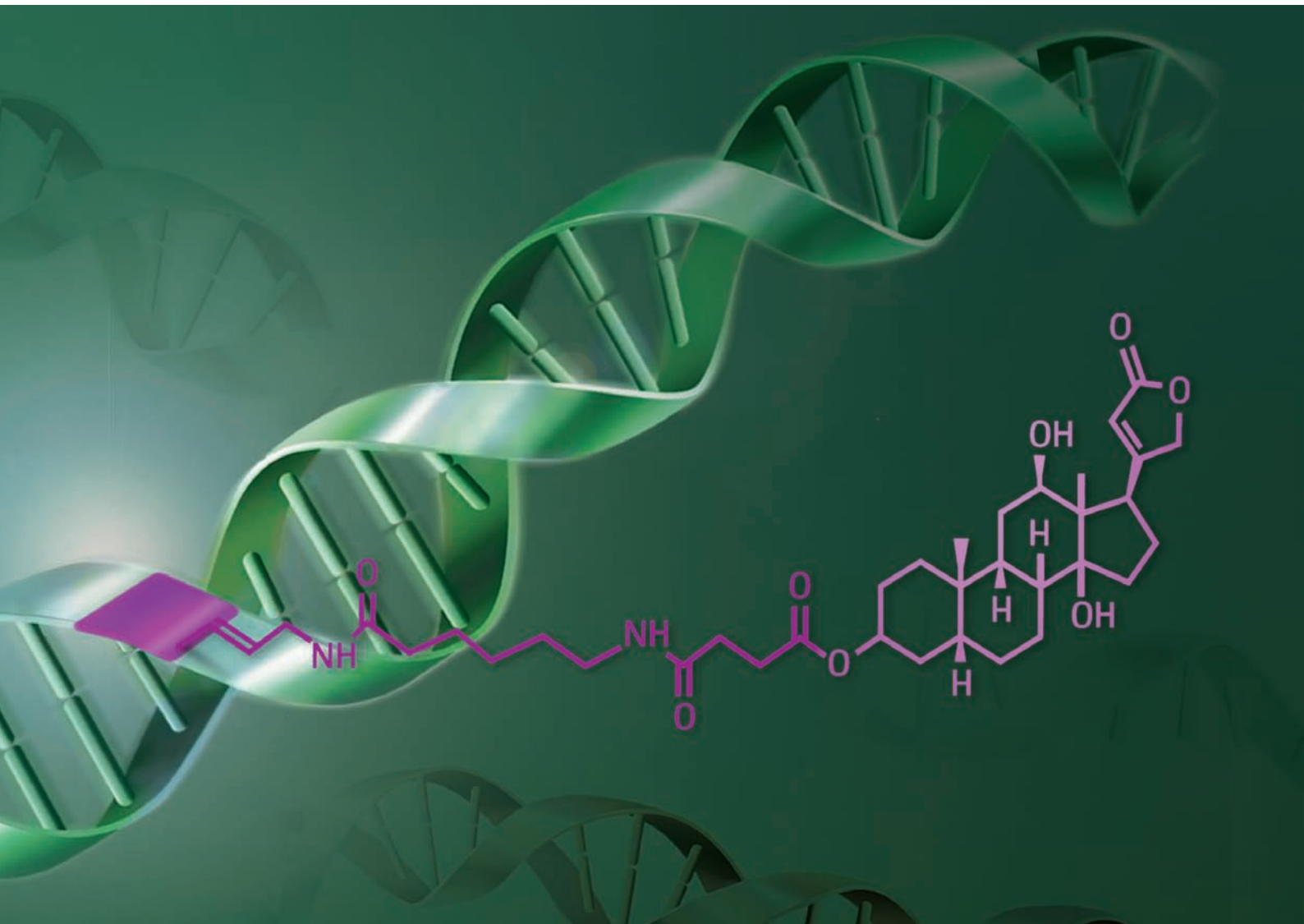
The DIG System

Labeling and Detection of Nucleic Acids



The DIG System
Specifically Label and Detect Nucleic Acids

Publishable results require high level specific detection and low background. Do your hybridizations have nonspecific signals and high background? The DIG System is ideal for nucleic acid labeling. Flexibly use colorimetric, luminescent or fluorescent signal detection. Achieve high sensitivity and low background in very short exposure times

- **Specificity:** DIG antibodies do not bind other substrates.
- **Versatility:** Use DIG labeled probes for filters and *in situ* hybridization.
- **Proven:** Thousands of publications show why DIG is superior to radioactivity.

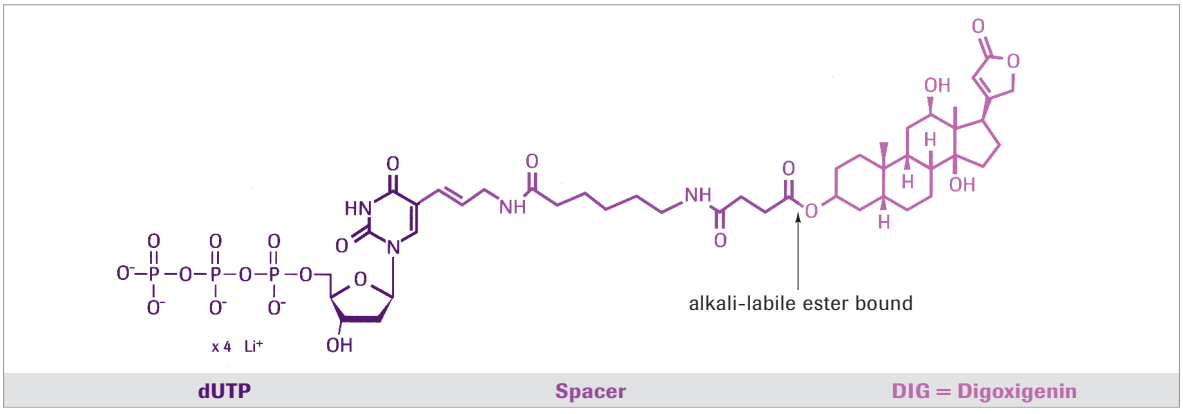


Figure 1: DIG-dUTP coupling is via an alkali-labile ester bond. Anti-DIG antibodies only bind to DIG, not other biological substrates. The alkali-labile ester bond ideal for filter stripping and reprobing.

Products are for life science research only. Not for use in diagnostic procedures.

Combine DIG Labeling with Established Protocols

High specificity and sensitivity are the reason, researchers worldwide choose the DIG System to detect nucleic acids using filter and in situ hybridization. Rely on the DIG System for straightforward, sensitive, and efficient nonradioactive labeling and detection. Use robust procedures and established protocols for low background and a high signal-to-noise ratio.

Probes for NA-labeling	Methods for Labeling	Hybridization and Detection
DIG labeling produces high sensitivity and low background. Easily label - DNA - RNA - oligonucleotides	Label the probe using familiar, efficient procedures. Quickly achieve superb results using PCR labeling or *in vitro* transcription.	The DIG System can be applied to filter or *in situ* hybridization. Established easy-to-use protocols make stripping and reprobing straightforward.

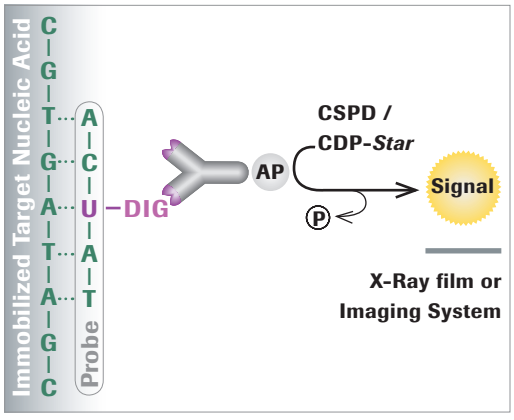


Figure 2: Example detecting DIG-labeled nucleic acids using chemiluminescence substrates. Immobilized target nucleic acids are hybridized to a DIG-labeled probe. Subsequent detection is performed using high affinity anti-digoxigenin antibodies coupled either to alkaline phosphatase (AP), horseradish peroxidase (POD), and fluorescein or rhodamine for colorimetric, chemiluminescence or fluorescence detection.

The DIG System
Find Applications and Corresponding Products

■ Blotting Applications

Simple, effective nonradioactive labeling and detection of nucleic acids ensures high signal-to-noise. All Kits are QC (quality control) tested, to function in different blot applications and guaranteed to be DNase and RNase free according to the current quality procedures. With DIG-labeled probes, you can easily detect single-copy genes on Southern blots, unique mRNAs on northern blots, or rare recombinants in bacterial colonies or viral plaques.

Labeling	Immobilization	Hybridization and Detection	
PCR	■ Nylon Membranes, positively charged	■ DIG Easy Hyb	■ Anti-Digoxigenin-AP, Fab fragments
■ DIG Probe Synthesis Kit	■ Buffers in a Box	■ Hybridization Bags	■ CDP-*Star*, ready-to-use
■ DIG Northern Starter Kit	■ Molecular Weight Marker, DIG-labeled (DNA or RNA)	■ Actin RNA Probe Labeled DIG (as control)	■ CDP-*Star*, ready-to-use NBT/BCIP
***In Vitro* Transcription**			■ DIG Wash and Block Buffer Set
■ DIG RNA Labeling Kit (SP6/T7)			■ Lumi Film
■ DIG RNA Labeling Mix			

■ Products are recommended but not absolutely required.

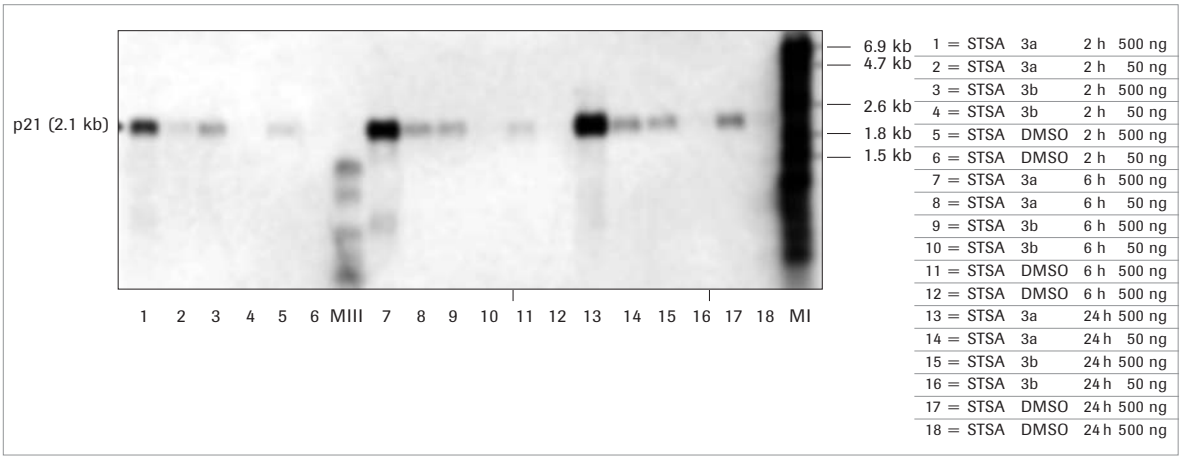


Figure 3: Northern blot hybridized with a DIG-labeled p21probe.
A strong increase over time is visible for the p21 mRNA. Exposure time: 3 minutes after 2 hours incubation with CDP-Star.

➜ Find additional information
Obtain Cancer Research Application Note No. 10,
DIG Application Manual for Filter Hybridization,
Catalog Number 05 353 149 001

In Situ Applications

In situ hybridization techniques allow specific nucleic acid sequences to be detected in morphologically preserved chromosomes, cells, or tissue sections. In combination with immunocytochemistry, *in situ* hybridization can relate microscopic topological information to gene activity at the DNA, mRNA, and protein levels. The DIG System can be used for fresh-frozen or fixed tissue and cells to detect expression patterns or chromosome aberrations.

Labeling	Immobilization	Hybridization and Detection
Nick Translation ■ Nick Translation Mix ■ DIG-Nick Translation Mix **PCR** ■ PCR DIG Probe Synthesis Kit ***In Vitro* Transcription** ■ DIG RNA Labeling Kit (SP6/T7) ■ DIG RNA Labeling Mix	**Tissue Preparation** depending on your laboratory application - FFPE - Fresh Frozen - Whole Mounts - Chromosome Spreads	■ DIG Wash and Block Buffer Set ■ Anti DIG AP Antibody, Fab fragments ■ Anti-Digoxigenin-Fluorescein, Fab fragments ■ Anti-Digoxigenin-Rhodamine, Fab fragments ■ Anti-Digoxigenin-POD, Fab fragments ■ NBT/BCIP/HNPP ■ POD conjugates

The DIG System can also be used in sophisticated micro-RNA staining techniques using common model organisms.

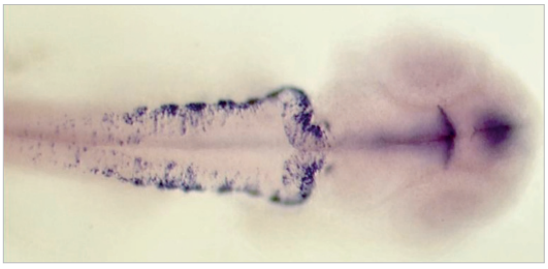


Figure 4: Dorsal view of a zebrafish embryo whole mount *in situ* hybridization. The slide was stained with a DIG-labeled RNA probe for the proneural transcription factor atoh1b and detected with NBT/BCIP.

➜ Find additional information
in the DIG Application Manual for Nonradioactive *In Situ* Hybridization, 4th Edition, Catalog Number 05 353 122 001

Additional Applications

Following nonradioactive techniques go beyond standard nucleic acid labeling and detection. Highly efficient and sensitive techniques include:

- **Glycan labeling**
- **Protein labeling**
- **Gel Shift assay**
- **PCR ELISA**
- **Telomere assay**

➜ Find additional information
about nonradioactive techniques for protein labeling, western blotting, and other applications,
www.roche-applied-science.com

Ordering Information

Kits for Labeling and Detection	Catalog Number	Pack Size
DIG-High Prime DNA Labeling and Detection Starter Kit I	11 745 832 910	1 kit (12 labeling reactions and 24 detection reactions)
DIG-High Prime DNA Labeling and Detection Starter Kit II	11 585 614 910	1 kit (12 labeling reactions and 24 detection reactions)
DIG DNA Labeling and Detection Kit	11 093 657 910	1 kit (25 labeling reactions and 50 blots)
DIG Northern Starter Kit	12 039 672 910	1 kit (10 labeling reactions and detection of 10 blots 10 x 10 cm^2)
Kits for Labeling		
PCR DIG Probe Synthesis Kit	11 636 090 910	1 kit (25 labeling reactions)
DIG DNA Labeling Kit	11 175 033 910	1 kit (40 labeling reactions)
DIG-High Prime	11 585 606 910	160 µl (40 labeling reactions)
DIG RNA Labeling Kit (SP6/T7)	11 175 025 910	1 kit (2 x 10 labeling reactions)
DIG Oligonucleotide 3'-End Labeling Kit, 2nd generation	03 353 575 910	1 kit (25 labeling reactions)
DIG Oligonucleotide 5'-End Labeling Set	11 480 863 001	1 set (10 labeling reactions)
DIG Oligonucleotide Tailing Kit, 2nd generation	03 353 583 910	1 kit (25 tailing reactions)
Mixes for Labeling		
DIG DNA Labeling Mix	11 277 065 910	50 µl (25 reactions)
DIG-High Prime	11 585 606 910	160 µl (40 reactions)
PCR DIG Labeling Mix**	11 585 550 910	2 x 250 µl (2 x 25 reactions)
DIG RNA Labeling Mix	11 277 073 910	40 µl (20 reactions)
Biotin-High Prime	11 585 649 910	100 µl (25 reactions)
Biotin RNA Labeling Mix	11 685 597 910	40 µl (20 reactions)
Fluorescein-High Prime*	11 585 622 910	100 µl (25 reactions)
PCR Fluorescein Labeling Mix	11 636 154 910	10 PCR reactions (100 µl each)
Fluorescein RNA Labeling Mix	11 685 619 910	40 µl (20 reactions)
Nick Translation Mix*	11 745 808 910	200 µl
DIG-Nick Translation Mix*	11 745 816 910	160 µl
Biotin-Nick Translation Mix*	11 745 824 910	160 µl
Nucleotides for Labeling		
Digoxigenin-11-dUTP, alkali-labile	11 573 152 910 11 573 179 910	25 nmol (25 µl) 125 nmol (125 µl)
Digoxigenin-11-dUTP, alkali-stable	11 093 088 910 11 558 706 910 11 570 013 910	25 nmol (25 µl) 125 nmol (125 µl) 5 x 125 nmol (5 x 125 µl)
Digoxigenin-11-UTP	11 209 256 910 03 359 247 910	250 nmol (25 µl) 200 nmol (57 µl)
Digoxigenin-11-ddUTP	11 363 905 910	25 nmol (25 µl)
Biotin-16-dUTP	11 093 070 910	50 nmol (50 µl)
Biotin-16-UTP	11 388 908 910	250 nmol (25 µl)
Biotin-16-ddUTP	11 427 598 910	25 nmol (25 µl)
Fluorescein-12-dUTP*	11 373 242 910	25 nmol (25 µl)
Fluorescein-12-UTP*	11 427 857 910	250 nmol (25 µl)
Tetramethyl-rhodamine-5-dUTP*	11 534 378 910	25 nmol (25 µl)
Kits for Detection		
DIG Luminescent Detection Kit	11 363 514 910	1 kit (50 blots)
DIG Nucleic Acid Detection Kit	11 175 041 910	1 kit (40 blots)
Anti-DIG Antibody for Detection on Membranes		
Anti-Digoxigenin-AP, Fab fragments	11 093 274 910	150 U (200 µl)
Anti-Digoxigenin-Fluorescein, Fab fragments*	11 207 741 910	200 µg
Anti-Digoxigenin-Rhodamine, Fab fragments*	11 207 750 910	200 µg
Anti-Digoxigenin-POD, Fab fragments	11 207 733 910	150 U

Chemiluminescent Substrates for Alkaline Phosphatase	Catalog Number	Pack Size
CDP-*Star*	11 685 627 001 11 759 051 001	1 ml 2 x 1 ml
CDP-*Star*, ready-to-use	12 041 677 001	2 x 50 ml
CSPD	11 655 884 001	1 ml
CSPD, ready-to-use	11 755 633 001	2 x 50 ml
Chromogenic Substrates for Alkaline Phosphatase		
BM purple*	11 442 074 001	100 ml
5-Bromo-4-chloro-3-indolyl-phosphate (BCIP)*	11 383 221 001	3 ml (150 mg)
HNPP Fluorescent Detection Set*	11 758 888 001	1 set (5 mg HNPP, 100 mg Fast Red TR)
NBT (4-Nitroblue tetrazolium chloride)*	11 383 213 001	3 ml (300 mg)
NBT/BCIP Ready-to-Use Tablets*	11 697 471 001	20 tablets
NBT/BCIP Stock Solution*	11 681 451 001	8 ml
Fast Red Tablets*	11 496 549 001	20 tablets
Molecular Weight Markers, DIG-labeled		
DNA Molecular Weight Marker II, DIG-labeled	11 218 590 910	5 µg (500 µl)
DNA Molecular Weight Marker III, DIG-labeled	11 218 603 910	5 µg (500 µl)
DNA Molecular Weight Marker VI, DIG-labeled	11 218 611 910	5 µg (500 µl)
DNA Molecular Weight Marker VII, DIG-labeled	11 669 940 910	5 µg (500 µl)
DNA Molecular Weight Marker VIII, DIG-labeled	11 449 451 910	5 µg (500 µl)
RNA Molecular Weight Marker I, DIG-labeled	11 526 529 910	4 µg (200 µl)
RNA Molecular Weight Marker II, DIG-labeled	11 526 537 910	2 µg (200 µl)
RNA Molecular Weight Marker III, DIG-labeled	11 373 099 910	2 µg (200 µl)
Nucleic Acids and Probes, DIG-labeled		
Actin RNA Probe, DIG-labeled	11 498 045 910	2 µg
DIG-labeled Control DNA	11 585 738 910	50 µl
DIG-labeled Control RNA	11 585 746 910	50 µl
Additonal Kits for Nonradioactive Analysis		
PCR ELISA, DIG Detection	11 636 111 910	192 detection reactions
DIG Gel Shift Kit, 2nd generation	03 353 591 910	1 kit
Lumi-Light^{PLUS} Western Blotting Kit	12 015 218 001	1 kit
TeloTAGGG Telomere Length Assay	12 209 136 001	1 kit
Membranes and Films for Nonradioactive Analysis		
Nylon Membranes, positively charged	11 209 272 001 11 209 299 001 11 417 240 001	10 sheets (20 x 30 cm) 20 sheets (10 x 15 cm) 1 roll (0.3 x 3 m)
Nylon Membranes for Colony and Plaque Hybridization	11 699 075 001 11 699 083 001	50 discs (each 82 mm diameter) 50 discs (each 132 mm diameter)
Lumi-Film Chemiluminescent Detection Film	11 666 916 001 11 666 657 001	100 films (7.1 x 9.4 inches 18 x 24 cm) 100 films (8 x 10 inches 20.3 x 25.4 cm)
Hybridization Bags	11 666 649 001	50 bags
Additional Reagents for Nonradioactive Analysis		
DIG Wash and Block Buffer Set	11 585 762 001	1 set (30 blots)
DIG Easy Hyb	11 603 558 001	500 ml
DIG Easy Hyb Granules	11 796 895 001	Granules for 6 x 100 ml
Buffers in a Box, SSC, 20x	11 666 681 001	4 l
Blocking Reagent	11 096 176 001	50 g
DNA, MB-grade (fish sperm)*	11 467 140 001	500 mg (50 ml)
COT Human DNA	11 581 074 001	500 µg (500 µl)
tRNA, RNase negative*	10 109 541 001 10 109 550 001	100 mg 500 mg
RNA, from yeast*	10 109 223 001	100 g
Hexanucleotide Mix	11 277 081 001	100 µl (50 reactions)
Digoxigenin-3-O-methylcarbonyl-ε-aminocaproic-acid-N-hydroxy-succinimide ester	11 333 054 001	5 mg
DAB Substrate*	11 718 096 001	1 pack

* Recommended for *in situ* hybridization.

** Recommended for direct detection.

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