

# Anti-Digoxigenin-AP, Fab fragments

For the detection of digoxigenin-labeled compounds

**Cat. No. 11 093 274 910** 150 U (200 µl)

Solution, stabilized

**Version December 2005**

Store at 2-8° C

## Product characteristics

<b>Antibody type</b>	Fab fragments from an anti-digoxigenin antibody from sheep, conjugated with alkaline phosphatase (AP).
<b>Specificity</b>	The Fab fragments bind to digoxigenin.
<b>Antibody production</b>	After immunization with digoxigenin the sheep IgG was purified by ion exchange chromatography and the specific IgG was isolated by immunosorption. The Fab fragments obtained by papain digestion were conjugated with AP and stabilized in 50 mM triethanolamine buffer, 3 mM NaCl, 1 mM MgCl <sub>2</sub> , 0.1 mM ZnCl <sub>2</sub> , 1% bovine serum albumin (w/v), pH 7.6.
<b>Applications</b>	<p>The conjugates can be used for the detection of digoxigenin-labeled nucleic acids (DNA; RNA) and proteins, e.g. glycoproteins in the following procedures:</p> <ul style="list-style-type: none"> <li>• Southern blots</li> <li>• Northern blots</li> <li>• colony- or plaque-hybridizations</li> <li>• nonradioactive DNA sequencing blots.</li> <li>• Gel shift assays</li> <li>• RNase protection assay</li> <li>• cDNA array detection</li> <li>• immunoblotting</li> <li>• histochemistry</li> <li>• ELISA</li> <li>• <i>in situ</i> hybridization</li> </ul>

<b>Storage/ Stability</b>	<p>The undiluted conjugate is stable at 2 to 8°C through the expiration date printed on the label.</p> <p><b>Note:</b> Do not freeze!</p>
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## Antibody dilution

<b>Antibody concentration</b>	The antibody concentration depends on application and substrate used for the detection of the antibody-conjugate.
<b>Antibody dilution</b>	<p>Before dilution centrifuge the antibody for 5 min full speed in the original vial prior to each use, and pipet the necessary amount carefully from the surface.</p> <p><b>Note:</b> The diluted antibody is stable at 2-8°C for 12 h.</p> <p><b>Always prepare fresh!</b></p>

## Buffers for dilution

In the following table please find the recommended buffers for the dilution of anti-digoxigenin-AP:

<b>Membrane applications</b>	<ul style="list-style-type: none"> <li>• Detection of DIG-labeled DNA/ RNA: 1x Blocking solution: 1% Blocking reagent (w/w) in Maleic acid buffer (100 mM Maleic acid, 150 mM NaCl, pH 7.5).</li> <li>• Detection of DIG-labeled Glycoproteins: 1x TBS: Tris Buffered Saline (50 mM Tris, 150 mM NaCl, pH 7.5)</li> </ul>
<b>other applications</b>	<p>100 mM Tris-HCl, 150 mM NaCl, pH 7.5</p> <p>If necessary the following reagents can be used for the reduction of unspecific binding:</p> <ul style="list-style-type: none"> <li>• 1 % Blocking reagent (w/v) (Cat. No. 1 096 176),</li> <li>• 1-5% heat inactivated fetal calf serum (FCS) (v/v)</li> <li>• sheep normal serum</li> </ul>

## DNA/RNA blot application

<b>DNA/RNA Blot application</b>	<p>Nucleic acid probes can be labeled very efficiently with digoxigenin (DIG) and be used as hybridization probes in various membrane blot applications. After stringency washes, the blots are subjected to immunological detection using anti-digoxigenin antibody conjugated to alkaline phosphatase and a chemiluminescent or color substrate. Detailed protocols for DIG labeling and hybridization are available in the product descriptions of various DIG labeling and detection kits (see below) and the DIG Application Manual.</p>
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<b>Detection with chemiluminescent substrates</b>	<p>Enzymatic dephosphorylation of CSPD or CDP-<i>Star</i> by alkaline phosphatase leads to a light emission which is recorded on X-ray film or imaging device.</p>
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CSPD and CDP-*Star* can be used for the detection of alkaline phosphatase conjugates either in solution or on solid supports. It is especially suited for highly sensitive and fast detection of nonradioactively labeled nucleic acids in various types of blotting applications.

**Note:** For chemiluminescent detection nylon membranes\* should be used for blotting of nucleic acids.

\* available from Roche Applied Science

**Detection with NBT/BCIP**

Colorimetric detection of DIG-labeled probe is usually performed with two colorless substrates referred to as BCIP and NBT which form a redox system. BCIP is oxidized by alkaline phosphatase to indigo by release of a phosphate group. In parallel, NBT is reduced to diformazan. The reaction products form a water insoluble dark blue to brownish precipitate, depending on the type of membrane.

**Antibody concentration**

The anti-DIG-AP should be diluted as described in the following table. Incubate at 15–25°C. Detailed protocols using color and chemiluminescent detection are available in the pack inserts of our DIG Kits (please compare to the ordering information).

Detection of nucleic acids on blots with:	Dilution in Blocking solution	anti-DIG-AP Concentration	Volume for 100 cm <sup>2</sup>
CSPD	1: 10 000	75 mU/ml	20 ml
CDP- <i>Star</i>	1: 10 000–1: 20 000	75 mU/ml – 37.5 mU/ml	20 ml
NBT/BCIP	1: 5 000	150 mU/ml	20 ml

**Other applications****Working concentration**

Please refer to the following table for recommended concentrations:

Application	Dilution	Conc. [mU/ml]	Sufficient for...
<i>in situ</i> hybridization	1:100–1:500	7500 – 1500	400 – 2000 <i>in situ</i> hybridizations
Detection of sugars in glyco-conjugates	1:1000	750	20 blots
Immunoblotting	1:1500–1:3000	500 – 250	30 – 60 blots
Immuno-histochemistry	1:1500–1:3000	500 – 250	6000 – 12000 sections
ELISA	1:2500–1:5000	300 – 150	2500 – 5000 tests

**Ordering Information**

Roche Applied Science offers a large selection of reagents and systems for life science research. For a complete overview of related products and manuals, please visit and bookmark our homepage <http://www.roche-applied-science.com> and our Special Interest Sites including:

- DIG Reagents and Kits for Non-Radioactive Nucleic Acid Labeling and Detection: <http://www.roche-applied-science.com/DIG/>

**Kits**

Product	Pack size	Cat. No.
DIG DNA Labeling and Detection Kit	25 labeling reactions and 50 blots	11 093 657 910
DIG DNA Labeling Kit	40 labeling reactions	11 175 033 910
DIG Gel Shift Kit 2nd Generation	1 kit	03 353 591 910
DIG Glycan Detection Kit	Kit for 100 labeling reactions and 25 filter detections of 100 cm <sup>2</sup> each	11 142 372 001
DIG Glycan Differentiation Kit	1 kit	11 210 238 001
DIG High Prime Labeling and Detection Starter Kit I	12 labeling reactions and 24 blots (10× 10 cm)	11 745 832 910
DIG High Prime Labeling and Detection Starter Kit II	12 labeling reactions and 24 blots	11 585 614 910
DIG Luminescent Detection Kit for Nucleic acids	1 kit (50 blots)	11 363 514 910
DIG Northern Starter Kit	1 kit (10 labeling reactions)	12 039 672 910
DIG Nucleic Acid Detection Kit	40 blots (10× 10 cm)	11 175 041 910
DIG PCR Probe Synthesis Kit	25 reactions	11 636 090 910

**Single reagents**

Product	Pack size	Cat. No.
Blocking reagent	50 g	11 096 176 001
CDP- <i>Star</i>	1 ml 2× 1 ml	11 685 672 001 11 759 051 001
CDP- <i>Star</i> , ready -to-use	2× 50 ml	12 041 677 001
CSPD	1 ml 2× 1 ml 4× 1 ml	11 655 884 001 11 759 035 001 11 759 043 001
CSPD , ready-to-use	2× 50 ml	11 755 633 001
DIG Easy Hyb (ready-to-use hybridization solution without formamide)	500 ml	11 603 558 001
DIG Easy Hyb Granules	1 set (6× 100 ml)	11 796 895 001
DIG Wash and Block Buffer Set	30 blots (100 cm <sup>2</sup> )	11 585 762 001
Hybridization bags	50 bags	11 666 649 001
Lumi-Film Chemiluminescent Detection Film	100 films (18× 24 cm) 100 films (20.3× 25.4 cm)	11 666 916 001 11 666 657 001
NBT/BCIP stock solution	8 ml	11 681 451 001
Nylon Membrane, positively charged (20× 30 cm) (10× 15 cm) (0.3× 3 m roll)	10 sheets 20 sheets 1 roll	11 209 272 001 11 209 299 001 11 417 240 001
Nylon Membranes for Colony/Plaque Hybridization	50 discs (each 82 mm diameter) 50 discs (each 132 mm diameter)	11 699 075 001 11 699 083 001

**Available printed material**

DIG Appl. Manual for Filter Hybridization
Nonradioactive In situ Hybridization Manual
DIG Product Selection Guide
Lab FAQs

The labeling of nucleic acids with DIG is covered by EP patents 0 324 474 and 0 371 262 as well as the following US patents 5,344,757, 5,354,657 and 5,702,888 owned by Roche Diagnostics GmbH.

**Contact and Support**

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site** at:

**[www.roche-applied-science.com/support](http://www.roche-applied-science.com/support)**

To call, write, fax, or email us, visit the Roche Applied Science home page, [www.roche-applied-science.com](http://www.roche-applied-science.com), and select your home country. Country-specific contact information will be displayed. Use the Product Search function to find Pack Inserts and Material Safety Data Sheets.



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