Print

# 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 17
Content version: April 2016

Store at +2 to +8°C

## Product characteristics

**Antibody type**
Fab fragments from an anti-digoxigenin antibody from sheep, conjugated with alkaline phosphatase (AP).

**Specificity**
The Fab fragments bind to digoxigenin.

**Antibody production**
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 $MgCl_2$, 0.1 mM $ZnCl_2$, 1% bovine serum albumin (w/v), pH 7.6.

**Applications**
The conjugates can be used for the detection of digoxigenin-labeled nucleic acids (DNA; RNA) and proteins, e.g. glycoproteins in the following procedures:

- Southern blots
- northern blots
- colony- or plaque-hybridizations
- nonradioactive DNA sequencing blots.
- Gel shift assays
- RNase protection assay
- cDNA array detection
- immunoblotting
- histochemistry
- ELISA
- *in situ* hybridization

**Storage/ Stability**
The undiluted conjugate is stable at +2 to +8°C through the expiration date printed on the label.

***Note***: Do not freeze!

## Antibody dilution

**Antibody concentration**
The antibody concentration depends on application and substrate used for the detection of the antibody-conjugate.

**Antibody dilution**
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.

***Note:*** The diluted antibody is stable at +2 to +8°C for 12 h. **Always prepare fresh!**

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

| | |
|---|---|
| **Membrane applications** | • 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).<br>• Detection of DIG-labeled Glycoproteins: 1x TBS: Tris Buffered Saline (50 mM Tris, 150 mM NaCl, pH 7.5) |
| **other applications** | 100 mM Tris-HCl, 150 mM NaCl, pH 7.5<br>If necessary the following reagents can be used for the reduction of unspecific binding:<br>• 1 % Blocking reagent (w/v) (Cat. No. 1 096 176),<br>• 1-5% heat inactivated fetal calf serum (FCS) (v/v)<br>• sheep normal serum |

## DNA/RNA blot application

**DNA/RNA Blot application**
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.

**Detection with chemiluminescent substrates**
Enzymatic dephosphorylation of CSPD or CDP-Star by alkaline phosphatase leads to a light emission which is recorded on X-ray film or imaging device.

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 Diagnostics

**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 to be diluted as described in the following table. Incubate at +15 to +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 cm² |
|---|---|---|---|
| CSPD | 1: 10 000 | 75 mU/ml | 20 ml |
| CDP-Star | 1: 10 000-<br>1: 20 000 | 75 mU/ml -<br>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... |
|---|---|---|---|
| *in situ* hybridization | 1:100-<br>1:500 | 7500 -<br>1500 | 400 - 2000 *in situ* hybridizations |
| Detection of sugars in glycoconjugates | 1:1000 | 750 | 20 blots |
| Immunoblotting | 1:1500-<br>1:3000 | 500 - 250 | 30 - 60 blots |
| Immunohistochemistry | 1:1500-<br>1:3000 | 500 - 250 | 6000 - 12000 sections |
| ELISA | 1:2500-<br>1:5000 | 300 - 150 | 2500 - 5000 tests |

## Ordering Information

**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 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-Star | 1 ml<br>2× 1 ml | 11 685 627 001<br>11 759 051 001 |
| CDP-Star, ready -to-use | 2× 50 ml | 12 041 677 001 |
| CSPD | 1 ml | 11 655 884 001 |
| CSPD , ready-to-use | 2× 50 ml | 11 755 633 001 |
| DIG Easy Hyb solution (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 $cm^2$) | 11 585 762 001 |
| Hybridization bags | 50 bags | 11 666 649 001 |
| Lumi-Film Chemiluminescent Detection Film | 100 films (18× 24 cm)<br>100 films (20.3× 25.4 cm) | 11 666 916 001<br>11 666 657 001 |
| NBT/BCIP stock solution | 8 ml | 11 681 451 001 |
| Nylon Membrane, positively charged<br>(20× 30 cm)<br>(10× 15 cm)<br>(0.3× 3 m roll) | <br>10 sheets<br>20 sheets<br>1 roll | <br>11 209 272 001<br>11 209 299 001<br>11 417 240 001 |
| Nylon Membranes for Colony/ Plaque Hybridization | 50 discs (each 82 mm diameter) | 11 699 075 001 |

**Changes to Previous Version**

Editorial changes

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DIG EASY HYB is a trademark of Roche.
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