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

Anti-Digoxigenin-AP, Fab fragments
from sheep

Version: 18
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For the detection of digoxigenin-labeled compounds.
Solution, stabilized

Cat. No. 11 093 274 910	150 U 200 µl

Store conjugate at +2 to +8°C.

1. General Information

1.1. Contents

Vial / Bottle	Label	Content
1	Anti-Digoxigenin-AP, Fab fragments	1 vial, 200 µl, 150 U

1.2. Storage and Stability

Storage Conditions (Product)

When stored at +2 to +8°C, the conjugate is stable through the expiry date printed on the label.

Vial / Bottle	Label	Storage
1	Anti-Digoxigenin-AP, Fab fragments	Store at +2 to +8°C. ⚠ ***Do not freeze.***

Storage Conditions (Working Solution)

The diluted antibody is stable at +2 to +8°C for 12 hours.
⚠ ***Always prepare fresh.***

1.3. Additional Equipment and Reagent required

For Chemiluminescent Detection

- CSPD*
- CDP-*Star**
- Nylon Membranes, positively charged*
- Blocking Reagent*
- Lumi-Film Chemiluminescent Detection Film*

For Colorimetric Detection

- NBT/BCIP Stock Solution*
- Blocking Reagent*

1.4. Application

The conjugate can be used for the detection of digoxigenin-labeled compounds, such as:
- Digoxigenin-labeled nucleic acids (DNA, RNA)
- Proteins such as glycoproteins

Applications include:
- Southern blots, northern blots
- Colony or plaque hybridizations
- Nonradioactive DNA sequencing blots
- Gel shift assays
- RNase protection assays
- cDNA array detection
- Immunoblotting
- Histochemistry
- ELISA
- *In situ* hybridization

2. How to Use this Product

2.1. Before you Begin

General Considerations

DNA and RNA Blot Applications

Nucleic acid probes can be labeled very efficiently with digoxigenin and be used as hybridization probes in various membrane blot applications. After stringency washes, the blots are subjected to immunological detection using a anti-digoxigenin antibody conjugated to alkaline phosphatase and a chemiluminescent or color substrate. Detailed protocols for DIG labeling and hybridization are available in the Instructions for use of the various kits.

Detection with Chemiluminescent Substrates

Enzymatic dephosphorylation of CSPD* or CDP-*Star** by alkaline phosphatase leads to a light emission which can be recorded on X-ray film or Lumi-Film*, or an 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.

For chemiluminescent detection, Nylon Membranes should be used for blotting of nucleic acids.*

Detection with Colorimetric Substrates

Colorimetric detection of a DIG-labeled probe is usually performed with the two colorless substrates BCIP and NBT. These substrates 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.

Working Solution

Dilution Buffers

Recommended buffers for the dilution of Anti-Digoxigenin-AP, Fab fragments are shown in the following table:

Membrane Applications	Other Applications
Detection of DIG-labeled DNA/RNA ▪ 1x Blocking solution: 1% Blocking Reagent (w/v) in maleic acid buffer (100 mM maleic acid, 150 mM NaCl, pH 7.5) **Detection of DIG-labeled Glycoproteins** ▪ 1x TBS: Tris-buffered saline (50 mM Tris, 150 mM NaCl, pH 7.5)	100 mM Tris-HCl, 150 mM NaCl, pH 7.5. If necessary, the following reagents can be used for the reduction of nonspecific binding: ▪ 1% Blocking Reagent* (w/v) ▪ 1 to 5% heat inactivated fetal calf serum (FCS) (v/v) ▪ Normal sheep serum

2.2. Parameters

Specificity

The Fab fragments bind to digoxigenin.

Working Concentration

Handling Instructions

Centrifuge the antibody for 5 minutes at full speed in the original vial prior to each use. Always pipette the necessary amount carefully from the surface.

Working Concentration DNA and RNA Blot Applications

The Anti-Digoxigenin-AP, Fab fragments should to be diluted as described in the following table. Incubate at +15 to +25°C. Detailed protocols using colorimetric and chemiluminescent detection are available in the Instructions for Use of the various DIG Kits.

The working concentration of antibody depends on the application and the substrate used for the detection of the antibody conjugate. The following concentrations should be taken as a guideline:

Detection of Nucleic Acids on Blots	Dilution in Blocking Solution	Anti-DIG-AP Concentration [mU/ml]	Volume for 100 cm² Membrane [ml]
CSPD	1:10,000	75	20
CDP-*Star*	1:10,000 – 1:20,000	75 – 37.5	20
NBT/BCIP	1:5,000	150	20

Working Concentration Other Applications

The working concentration of antibody depends on the application and the substrate used for the detection of the antibody conjugate. The following concentrations should be taken as a guideline:

Application	Dilution	Concentration [mU/ml]	Sufficient for:
In situ hybridization	1:100 – 1:500	7,500 – 1,500	400 – 2,000 *in situ* hybridizations
Detection of sugars in glycoconjugates	1:1,000	750	20 blots
Immunoblotting	1:1,500 – 1:3,000	500 – 250	30 – 60 blots
Immunohistochemistry	1:1,500 – 1:3,000	500 – 250	6,000 – 12,000 sections
ELISA	1:2,500 – 1:5,000	300 – 150	2,500 – 5,000 tests

3. Additional Information on this Product

3.1. Test Principle

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 alkaline phosphatase (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.

Antibody Type

Fab fragments from an anti-digoxigenin antibody from sheep, conjugated with alkaline phosphatase.

4. Supplementary Information

4.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

4.2. Changes to previous version

Layout changes.
Editorial changes.

4.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
Lumi-Film Chemiluminescent Detection Film	100 films, 7.1 x 9.4 inches, 18 x 24 cm, *Not available in US*	11 666 916 001
Blocking Reagent	50 g	11 096 176 001
CDP-*Star*, ready-to-use	2 x 50 ml	12 041 677 001
Nylon Membranes for Colony and Plaque Hybridization	50 discs, 82 mm diameter	11 699 075 001
Nylon Membranes, positively charged	10 sheets, 20 x 30 cm	11 209 272 001
	20 sheets, 10 x 15 cm	11 209 299 001
	1 roll, 0.3 x 3 m	11 417 240 001
NBT/BCIP Stock Solution	8 ml	11 681 451 001
CSPD, ready-to-use	2 x 50 ml	11 755 633 001

4.4. Trademarks

All product names and trademarks are the property of their respective owners.

4.5. License Disclaimer

For patent license limitations for individual products please refer to:
List of biochemical reagent products and select the corresponding product catalog.

4.6. Regulatory Disclaimer

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

4.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

4.8. Contact and Support

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

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed

0222.11213164001 ®

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim
Germany