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# DIG Nucleic Acid Detection Kit

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**Cat. No. 11 175 041 910**

Kit for the detection of 40 blots of 10 × 10 $cm^2$

Detection of digoxigenin-labeled nucleic acids by enzyme immunoassay and enzyme-catalyzed color reaction with NBT/BCIP

Store at −15 to −25°C

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# 1. Preface

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## 1.2 Kit contents

**Contents**

| Bottle/ Cap | Label | Contents |
|---|---|---|
| **1** | DIG-labeled Control DNA* | • 50 µl<br>• [5 µg/ml] pBR328 DNA (linearized with *Bam* HI)<br>• clear solution<br>• determination of labeling efficiency |
| **2** | DNA Dilution Buffer | • 1 ml<br>• [50 µg/ml fish sperm DNA in 10 mM Tris-HCl, 1 mM EDTA; pH 8.0 at +25°C]<br>• clear solution |
| **3** | Anti-Digoxigenin-AP conjugate* | • 200 µl<br>• [750 U/ml]<br>• polyclonal sheep anti-digoxigenin, Fab-fragments, conjugated to alkaline phosphatase<br>• clear solution |
| **4** | NBT/BCIP* | • 8 x 1 ml<br>• 50 x conc. stock solution [18.75 mg/ml nitroblue tetrazolium chloride and 9.4 mg/ml 5-bromo-4-chloro-3-indolyl-phosphate, toluidine salt in 67% (v/v) DMSO].]<br>• Can vary between light yellow and brown, clear solution<br>• Precipitating chromogenic substrate for alkaline phosphatase.<br>***Note:*** Store protected from light! The color of the stock solution can vary between light yellow and brown, depending on the lot used. The color does not impair the quality or the function of the substrate. |
| **5** | Blocking reagent* | 2 bottles, each with 50 g powder |

* *available from Roche Diagnostics*

# 2. Introduction

## 2.1 Product overview

**Test principle**

DIG-labeled DNA, RNA or oligonucleotide probes are detected, after hybridization to target nucleic acids, by enzyme-linked immunoassay using an antibody-conjugate (anti-digoxigenin alkaline phosphatase conjugate, anti-DIG-AP). A subsequent enzyme-catalyzed color reaction with 5-bromo-4-chloro-3-indolyl phosphate (BCIP) and nitroblue tetrazolium salt (NBT) produces an insoluble blue precipitate, which visualizes hybrid molecules.

**Colorimetric reaction**

BCIP (1) is the AP-substrate which reacts further after the dephosphorylation to give a dark-blue indigo dye as an oxidation product. NBT (2) serves herein as the oxidant and gives also a dark-blue dye. It intensites thereby the color and makes the detection more sensitive.

**Immunological detection**

Hybridized filters can be detected immediately after stringest washings or stored dry for later detection. After blocking of the membrane, binding of anti-DIG-AP to hybridized DIG-labeled nucleic acids occurs. The color reaction is initiated at alkaline pH by the addition of BCIP and NBT. A blue precipitate starts to form within a few minutes and continues up to three days. Typically, the reaction can be terminated when the color precipitate is clearly visible, which can vary from 1-24 hours. Background is usually not observed due to the use of highly specific antibody Fab-fragments and an efficient blocking step.

**Application**

- Southern blots
- Northern blots
- other nucleic acid blotting applications
- *in situ* hybridization applications.

**Sample material**

DIG labeled nucleic acids.

**Assay time**

This table lists the reaction time of the single steps

| Step | Reaction time |
|---|---|
| Immunological detection | 1.5 h |
| Color development | 0.5-16 h |

**Number of blots**

40 blots of 10 × 10 $cm^2$ can be detected.

**Kit storage/ stability**

The unopened kit is stable at −15 to −25°C until the expiration date printed on the label.
Shipping conditions on dry ice.
Once opened, please refer to the following table for proper storage.

| Kit component | Storage |
|---|---|
| Anti-Digoxigenin-AP Conjugate (vial 3) | +2 to +8°C, stable |
| NBT/BCIP (vial 4) | +2 to +8°C, stable<br>***Note:*** During shipment of the kit on dry ice, a precipitate may occur which is dissolved by briefly warming to +37°C |

**Sensitivity and specificity**

A single copy gene (tissue plasminogen activator, t-PA gene) is detected in a Southern blot of 1 µg digested human placenta DNA.

# 3. Procedures and required materials

## 3.1 Immunological detection

**Additional reagents required**

The following buffers and the Blocking solution are also available in the DIG Wash and Block Buffer Set* (see Ordering Information).

| Solution | Composition / Preparation | Storage/ stability | Use |
|---|---|---|---|
| Washing buffer | 0.1 M Maleic acid, 0.15 M NaCl; pH 7.5 (+20°C); 0.3% (v/v) Tween 20 | +15 to +25°C, stable | Removal of unbound antibody |
| Maleic acid buffer | 0.1 M Maleic acid, 0.15 M NaCl; adjust with NaOH (solid) to pH 7.5 (+20°C) | +15 to +25°C, stable | Dilution of Blocking solution |
| Detection buffer | 0.1 M Tris-HCl, 0.1 M NaCl, pH 9.5 (+20°C) | +15 to +25°C, stable | Adjustment of pH 9.5 |

**Preparation of kit working solutions**

In the following table the preparation of kit working solutions is described.

| Solution | Composition / Preparation | Storage/ stability | Use |
|---|---|---|---|
| Blocking reagent | Dissolve Blocking reagent (bottle 5) in Maleic acid buffer to a final concentration of 10% (w/v) with shaking and heating either on a heating block or in a microwave oven.<br>Autoclave stock solution. | +2 to +8°C or −15 to −25°C | Preparation of Blocking solution |
| Blocking solution | Prepare a 1x working solution by diluting 10x Blocking reagent 1 : 10 with Maleic acid buffer. | Always prepare fresh | Blocking of unspecific binding sites |
| Antibody solution | Centrifuge the antibody for 5 min at 10,000 rpm in the original vial prior to each use, and pipet necessary amount carefully from the surface. Dilute anti-digoxigenin-AP 1:5,000 (150 mU/ml) in Blocking solution. | 12 hours at +2 to +8°C | Binding to the DIG-labeled probe |
| Color substrate solution | Add 200 µl of NBT/BCIP stock solution (vial 4) to 10 ml of Detection buffer.<br>***Note:*** Store protected from light! | Always prepare fresh | Visualization of antibody-binding |

## 3.1 Immunological detection, *Continued*

**Procedure**

The following procedure describes the immunological detection for a 10 × 10 $cm^2$ blot.

| Step | Action |
|---|---|
| 1 | After hybridization and stringency washes, rinse membrane briefly in **Washing buffer**. |
| 2 | Incubate for 30 min in 100 ml **Blocking solution**. |
| 3 | Incubate for 30 min in 20 ml **Antibody solution**. |
| 4 | Wash 2 x 15 min with 100 ml **Washing buffer**. |
| 5 | Equilibrate 2-5 min in 20 ml **Detection buffer**. |
| 6 | Incubate membrane in 10 ml freshly prepared **Color substrate solution** in a appropriate container in the dark. **Do not shake** during color development.<br>***Note:*** The color precipitate starts to form within a few minutes and the reaction is usually complete after 16 h. The membrane can be exposed to light for short time periods to monitor color development. |
| 7 | Stop the reaction, when desired spot or band intensities are achieved with 50 ml of sterile double dist. water or with TE-buffer.<br>Results can be documented by photocopying the wet filter or by photography. |

**Storage of membrane**

Please refer to the following table.

| IF... | THEN... |
|---|---|
| you want to reprobe the membrane | the membrane should not dry off at any time, store in sealed blastic bag.<br>***Note***: If you want to maintain the color, store membranes in TE buffer; do not allow the membrane to dry. |
| you don´t want to reprobe | dry the membrane at +15 to +25°C for storage.<br>***Note:*** Color fades upon drying. To revitalize the color, soak the membrane in TE buffer. |

## 3.2 Stripping and reprobing of membranes

**Additional reagents required**

- Dimethylformamid (DMF)
- 0.2 N NaOH, 0.1% SDS (w/v)
- 2x SSC.

**Protocol**

Please refer to the following table.
***Note***: When stripping and rehybridization of blots is planned, the membrane should not dry off at any time.
**Caution:** Work under a fume hood

| Step | Action |
|---|---|
| **1** | • Heat DMF in a large glass beaker in a water bath under a fume hood to +50 to +60°C.<br>• Incubate the membranes in the heated DMF until the blue color precipitate is removed from the filter.<br>**Caution:** DMF is volatile and can be ignited above +67°C. |
| **2** | Rinse membrane briefly in double distilled water. |
| **3** | Wash for 2 × 20 min in 0.2 N NaOH, 0.1% SDS (w/v) at +37°C under constant agitation. |
| **4** | Equilibrate briefly in 2x SSC. |
| **5** | Air dry, or use directly for hybridization. |

## 4. Typical results

**Table 1**

**DNA hybridization:**
Sensitivity depends both on the concentration of labeled DNA in the Hybridization solution and on the duration of color reaction.

| **Concentration of labeled DNA (ng/ml)** | **0.5** | **2** | **5** | **10** | **20** | **30** | **50** |
|---|---|---|---|---|---|---|---|
| pg homologous DNA detectable after: | | | | | | | |
| 1 h | - | 10 | 5 | 2 | 1 | 1 | 0.5 |
| 3 h | 10 | 2 | 1 | 0.5 | 0.5 | 0.5 | 0.2 |
| 16 h | 2 | 0.5 | 0.2 | 0.1 | 0.1 | 0.05 | 0.05 |
| color reaction | | | | | | | |

**Table 2**

**RNA hybridization:**
Sensitivity depends both on the concentration of labeled RNA in the hybridization solution and on the duration of color reaction.

| **Concentration of labeled RNA (ng/ml)** | **2** | **5** | **10** | **20** | **50** | **100** | **200** |
|---|---|---|---|---|---|---|---|
| pg homologous RNA detectable after: | | | | | | | |
| 1 h | - | 10 | 3 | 3 | 1 | 0.3 | 0.3 |
| 3 h | 10 | 3 | 1 | 0.3 | 0.3 | 0.1 | 0.1 |
| 16 h | 3 | 1 | 0.3 | 0.1 | 0.1 | 0.03 | 0.03 |
| color reaction | | | | | | | |

# 5. Appendix

## 5.1 Troubleshooting

**Trouble shooting table**

| Problem | Possible cause | Recommendation |
|---|---|---|
| Low sensitivity | Inefficient probe labeling | • Check labeling efficiency. The labeling reaction can be upscaled. Prolong incubation time to overnight.<br>• Clean up template DNA by phenolization.<br>• Use only fragments < 5 kb or predigest with a restriction enzyme (e.g., four bp cutter)<br>• Make sure that template is efficiently denatured before labeling. |
| | Low probe concentration in the hybridization | • Increase probe concentration, but use not more than 25 ng/ml DNA probe or 100 ng/ml RNA probe.<br>• Check hybridization and washing conditions.<br>• Prolong hybridization time.<br>• Prolong color development to 16 h. |
| High background | Inefficient hybridization | • Recalculate hybridization temperature.<br>• Do not the allow the membrane to dry between prehybridization and hybridization.<br>• If you use plastic bags, remove all air bubbles prior to sealing. |
| | Wrong type of nylon membrane | Some types of nylon membrane may cause high background: use nylon membrane*, especially tested for the DIG-System from Roche. |
| | Inefficient blocking before immuno-assay | Prolong blocking and washing steps. |
| | Ineffective stringency washes | • Check temperature of stringency washes.<br>• Prewarm wash solution to correct temperature |
| | Special hints for immuno-assay | When using laboratory trays for the detection procedure, they should be rigorously cleaned before use. Anti-DIG-AP binding and color development should be done in separate trays. |

## Supplementary Information

### References


1 Horwitz, J. P. et al. (1966), *J. Med. Chem.* **9**, 47
2 Michal, G. *et al.* in Methods of Enzymatic Analysis (BergmeyerH. U. ed.) 3rd edition 1983, Vol **1**, 197.,


### Changes to previous version

Editorial changes.

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## 5.2 Ordering Information

**Kits**

| Product | Pack Size | Cat. No. |
|---|---|---|
| DIG DNA Labeling Kit | 40 labeling reactions | 11 175 033 910 |
| DIG RNA Labeling Kit (SP6/T7) | 2x 10 reactions | 11 175 025 910 |
| DIG Oligonucleotide 3'-End Labeling Kit, 2nd Gen. | 25 reactions | 03 353 575 910 |
| DIG Oligonucleotide Tailing Kit, 2nd Gen. | 25 reactions | 03 353 583 910 |
| DIG Oligonucleotide 5'-End Labeling Set | 10 reactions | 11 480 863 910 |
| DIG-High Prime DNA Labeling and Detection Starter Kit I | 1 kit (12 labeling reactions and 24 detection reactions) | 11 745 832 910 |
| DIG-High Prime DNA Labeling and Detection Starter Kit II | 1 kit (12 labeling reactions and 24 detection reactions) | 11 585 614 910 |
| DIG Northern Starter Kit | 1 kit (10 labeling reactions and detection of 10 blots of 10 x 10 $cm^2$ ) | 12 039 672 910 |
| DIG Luminescent Detection Kit (CSPD) | 50 blots | 11 363 514 910 |
| PCR DIG Probe Synthesis Kit | 1 kit (25 reactions) | 11 636 090 910 |

**Single reagents**

| Product | Pack Size | Cat. No. |
|---|---|---|
| Anti-digoxigenin-AP conjugate, Fab fragments | 150 U (200 µl) | 11 093 274 910 |
| NBT/BCIP stock solution | 8 ml | 11 681 451 001 |
| NBT/BCIP tablets | 20 tablets | 11 697 471 001 |
| DIG-High Prime | 160 µl (40 reactions) | 11 585 606 910 |
| DIG-DNA Labeling Mix | 50 µl (25 reactions) | 11 277 065 910 |
| DIG-11-dUTP, alkali-labile | 25 nmol (25 µl)<br>125 nmol (125 µl) | 11 573 152 910<br>11 573 179 910 |
| Digoxigenin-11-dUTP,<br>alkali-stable | 25 nmol (25 µl)<br>125 nmol (125 µl)<br>5 x 125 nmol (5 x 125 µl) | 11 093 088 910<br>11 558 706 910<br>11 570 013 1910 |
| DIG-11-UTP | 250 nmol (25 µl) | 11 209 256 910 |
| DIG-11-ddUTP | 25 nmol (25 µl) | 11 363 905 910 |
| DIG-labeled control RNA | 50 µl | 11 585 746 910 |
| DIG-labeled control DNA | 50 µl | 11 585 738 910 |
| DIG RNA Labeling Mix | 40 µl (20 reactions) | 11 277 073 910 |
| DIG-NHS Ester | 5 mg | 11 333 054 001 |
| DIG Easy Hyb | 500 ml | 11 603 558 001 |
| DIG Easy Hyb Granules | Granules for 6x 100 ml | 11 796 895 001 |
| DIG Wash and Block Buffer Set | 30 blots (10x10 $cm^2$) | 11 585 762 001 |
| Blocking Reagent | 50 g | 11 096 176 001 |
| Nylon membranes, positively charged | 10 sheets (20x30 $cm^2$)<br>20 sheets (10x15 $cm^2$)<br>1 roll (0.3 x 3 m) | 11 209 299 001<br>11 209 272 001<br>11 471 240 001 |
| Hybridization Bags | 50 bags | 11 666 649 001 |
| DNA, MB grade | 500 mg (50 ml) | 11 467 140 001 |

** available from Roche Diagnostics*

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Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim
Germany