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

DIG Wash and Block Buffer Set

Version: 14
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Washing, blocking, and detection buffers (10x conc.) for the immunological detection of DIG- (digoxigenin-) labeled probes

Cat. No. 11 585 762 001 1 set
30 blots (100 cm^2)

Store product at +2 to +8°C.

1. General Information

1.1. Contents

Vial / Bottle	Label	Function / Description	Content
1	DIG Wash and Block Buffer Set, Washing buffer, 10x conc.	▪ Maleic acid buffer, 10x conc. with 3 to 5% Tween 20 (v/v). ▪ For the washing of filters.	2 bottles, 500 ml
2	DIG Wash and Block Buffer Set, Maleic acid buffer, 10x conc.	▪ Clear solution after crystals are completely dissolved. ▪ For dilution of the Blocking solution.	1 bottle, 500 ml
3	DIG Wash and Block Buffer Set, Blocking solution, 10x conc.	▪ 10% Blocking reagent in Maleic acid buffer. ▪ For the blocking of nonspecific binding.	1 bottle, 500 ml
4	DIG Wash and Block Buffer Set, Detection buffer, 10x conc.	▪ Clear solution. ▪ 1 M Tris-HCl, pH 9.5, 1 M NaCl. ▪ For the adjustment of pH to 9.5.	1 bottle, 100 ml

1.2. Storage and Stability

Storage Conditions (Product)

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

Vial / Bottle	Label	Storage
1	Washing buffer, 10x conc.	Store at +2 to +8°C. ⚠ ***Upon storage, Tween 20 separates as a yellow/ brown layer of approximately 1 cm on top of the clear solution. Shake Washing buffer vigorously before use; the Washing buffer appears like a milky suspension.***
2	Maleic acid buffer, 10x conc.	Store at +2 to +8°C.
3	Blocking solution, 10x conc.	Store at +2 to +8°C initially; after first usage, store in aliquots at −15 to −25°C. ⚠ ***Upon storage, a thin, milky/creamy layer forms on top of the turbid brown solution. Shake vigorously before use.***
4	Detection buffer, 10x conc.	Store at +2 to +8°C.

1.3. Additional Equipment and Reagent required

For preparation of working solutions

See section, Working Solution for additional information on how to prepare solutions.

- Washing buffer
- Maleic acid buffer
- Blocking solution
- Detection buffer
- Double-distilled water

For preparation of hybridization buffers

- SDS*
- 5x SSC*
- Formamide*
- N-lauroylsarcosine (v/v)

For immunological detection

- Anti-Digoxigenin-AP*, Fab fragments
- CSPD*, ready-to-use or CSP-Star*, ready-to-use
- Hybridization Bags*
- Lumi-Film*

1.4. Application

The DIG Wash and Block Buffer Set is used at various stages of DIG hybridization and DIG detection.

2. How to Use this Product

2.1. Before you Begin

General Considerations

Hybridization and washing conditions

The hybridization conditions depend largely on the type of probe, such as DNA, RNA, or oligonucleotide, and are described in detail in the working procedures of the corresponding DIG kits*. Detailed working instructions and practical hints, concerning probe labeling with DIG, hybridization, and chemiluminescent or color detection, are described in these kits.

Safety Information

Laboratory procedures

- Handle all samples as if potentially infectious, using safe laboratory procedures. As the sensitivity and titer of potential pathogens in the sample material varies, the operator must optimize pathogen inactivation by the Lysis / Binding Buffer or take appropriate measures, according to local safety regulations.
- Do not eat, drink or smoke in the laboratory work area.
- Do not pipette by mouth.
- Wear protective disposable gloves, laboratory coats and eye protection, when handling samples and kit reagents.
- Wash hands thoroughly after handling samples and reagents.

Waste handling

- Discard unused reagents and waste in accordance with country, federal, state, and local regulations.
- Safety Data Sheets (SDS) are available online on dialog.roche.com, or upon request from the local Roche office.

Working Solution

Preparation of working solutions and hybridization buffers

Buffer	Preparation of Working Solution	Storage and Stability	For use in...
Washing buffer	Dilute the Washing buffer with double-distilled water to a 1x-concentrated solution.	Store at +15 to +25°C. ⚠ ***Shake vigorously before use.***	Removal of nonspecific bound antibody.
Maleic acid buffer	Dilute the Maleic acid buffer with double-distilled water to a 1x-concentrated solution. ▪ 10x Maleic acid buffer (Bottle 2) may contain needle-shaped crystals. After diluting the entire bottle of 10x Maleic acid buffer with double-distilled water to a 1x-concentrated solution, the crystals will dissolve immediately. No further action is required. ⚠ ***If instead, portions of Bottle 2 are used several times for the preparation of 1x Maleic acid buffer, dissolve crystals by incubating the 10x Maleic acid buffer at +37°C (up to overnight), until completely dissolved.***	Store at +15 to +25°C.	Dilution of Blocking solution.
Blocking solution	Dilute the Blocking solution, 10x conc. with 1x Maleic acid buffer to a 1x-concentrated solution.	⚠ ***Always prepare fresh.***	Blocking of nonspecific binding sites.
Detection buffer	Dilute the Detection buffer with double-distilled water to a 1x-concentrated solution.	Store at +15 to +25°C.	Adjustment of pH to 9.5 for the substrate reaction.

Preparation of antibody solutions

Detection Substrate	Preparation of Working Solution	Storage and Stability	For use in...
CSPD	▪ Centrifuge Anti-Digoxigenin-AP, Fab fragments in the original vial for 5 minutes at 10,000 rpm prior to each use. ▪ Pipette the necessary amount of antibody carefully from the surface. ▪ Dilute Anti-Digoxigenin-AP, Fab fragments 1:10,000 (75 mU/ml) in Blocking solution.	Store for 12 hours at +2 to +8°C.	Binding to the DIG-labeled probe.
CDP-*Star*	▪ Centrifuge Anti-Digoxigenin-AP, Fab fragments in the original vial for 5 minutes at 10,000 rpm prior to each use. ▪ Pipette the necessary amount of antibody carefully from the surface. ▪ Dilute Anti-Digoxigenin-AP, Fab fragments 1:20,000 (37.5 mU/ml) in Blocking solution.	Store for 12 hours at +2 to +8°C.	Binding to the DIG-labeled probe.

2.2. Protocols

Use of Blocking reagent in buffers

For the hybridization and pre-hybridization of filters with DIG-labeled probes, add Blocking reagent to the hybridization buffer.

Hybridization Buffer	Composition
Standard hybridization buffer	▪ 5x SSC* ▪ 0.1% N-lauroylsarcosine (w/v) ▪ 0.02% SDS* (w/v) ▪ 1% Blocking solution (v/v) (1/10 volume of Blocking solution, 10x conc.)
Standard hybridization buffer with Formamide	▪ 50% Formamide* (v/v) deionized ▪ 5x SSC* ▪ 0.1% N-lauroylsarcosine (w/v) ▪ 0.02% SDS* (w/v) ▪ 2% Blocking solution (v/v) (1/5 volume of Blocking solution, 10x conc.)
High SDS hybridization buffer	▪ 7% SDS*, ▪ 50% Formamide* (v/v) deionized ▪ 5x SSC* ▪ 50 mM sodium phosphate, pH 7.0 ▪ 0.1% N-lauroylsarcosine (v/v) ▪ 2% Blocking solution (w/v) (1/5 volume of Blocking solution, 10x conc.)

Immunological detection for blot applications

The following steps describe how to perform the immunological detection on a 100 cm^2 membrane.

⚠ ***Perform all incubations at +15 to +25°C with agitation. If the membrane is to be reprobed, do not allow the membrane to dry at any time.***

1. After hybridization and stringency washes, rinse membrane briefly for 1 to 5 minutes in Washing buffer.
   ⚠ ***Shake Washing buffer vigorously before use.***
2. Incubate for 30 minutes in 100 ml Blocking solution.
3. Incubate for 30 minutes in 20 ml Antibody solution.
4. Wash 2 × 15 minutes in 100 ml Washing buffer.
5. Equilibrate 3 minutes in 20 ml Detection buffer.
6. Place membrane with DNA/RNA side facing up on a development folder or Hybridization Bag* and apply 0.5 to 1 ml CSPD or CDP-*Star*, until the membrane is evenly soaked.
   – Immediately cover the membrane with the second sheet of the folder to spread the substrate evenly and without air bubbles over the membrane.
   – Incubate for 5 minutes at +15 to + 25°C.

7. Squeeze out excess liquid and seal the edges of the development folder.
⚠ ***Drying of the membrane during exposure will result in dark background.***

8. Depending on the detection substrate used, follow these steps:

Detection substrate	Step
CSPD	Incubate the damp membrane for 10 minutes at +37°C to enhance the luminescent reaction.
CDP-*Star*	Continue directly to Step 9.

9. Expose using an imaging instrument for 5 to 20 minutes, or to X-ray film or Lumi-Film* for 15 to 25 minutes at +15 to +25°C.

i When using X-ray films, multiple exposures can be taken to achieve the desired signal strength.

3. Additional Information on this Product

3.1. Quality Control

For lot-specific certificates of analysis, see section **Contact and Support**.

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.
Update to include new safety Information to ensure handling according controlled conditions.

4.3. Ordering Information

Product	Pack Size	Cat. No.
Consumables		
Hybridization Bags	50 bags, 25 cm x 23 cm	11 666 649 001
Reagents, kits		
Sodium Dodecyl Sulfate (SDS)	1 kg	11 667 289 001
Buffers in a Box, Premixed SSC Buffer, 20x	4 l	11 666 681 001
Formamide	500 ml	11 814 320 001
DIG DNA Labeling and Detection Kit	1 kit, 25 labeling reactions of 10 ng - 3 µg DNA and detection of 50 blots of 100 cm^2	11 093 657 910
DIG-High Prime DNA Labeling and Detection Starter Kit II	1 kit, 12 labeling reactions of 10 ng to 3 µg DNA and detection of 24 blots of 100 cm^2	11 585 614 910
CSPD, ready-to-use	2 x 50 ml	11 755 633 001
Lumi-Film Chemiluminescent Detection Film	100 films, 8 x 10 inches, 20.3 x 25.4 cm	11 666 657 001
DIG-High Prime DNA Labeling and Detection Starter Kit I	1 kit, 12 labeling reactions of 10 ng to 3 µg DNA and detection of 24 blots of 100 cm^2	11 745 832 910
DIG Northern Starter Kit	1 kit, 10 labeling reactions and detection of 10 blots of 10 x 10 cm^2	12 039 672 910
CDP-*Star*, ready-to-use	2 x 50 ml	12 041 677 001
DIG Nucleic Acid Detection Kit	1 kit, Detection of 40 blots of 10 cm x 10 cm	11 175 041 910
DIG RNA Labeling Kit (SP6/T7)	1 kit, 2 x 10 labeling reactions	11 175 025 910
DIG Luminescent Detection Kit	1 kit, 50 blots with a size of 10 x 10 cm^2	11 363 514 910
Anti-Digoxigenin-AP, Fab fragments	150 U, 200 µl	11 093 274 910

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.

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.

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