

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



Blocking Reagent

For nucleic acid hybridization and detection

 **Version: 18**
Content Version: May 2021

Cat. No. 11 096 176 001 50 g

Store product at +15 to +25°C.

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1. General Information

1.1. Contents

Vial / Bottle	Label	Function / Description	Content
1	Blocking Reagent	Powder	1 bottle, 50 g

1.2. Storage and Stability

Storage Conditions (Product)

When stored at +15 to +25°C, the product is stable through the expiry date printed on the label.

Vial / Bottle	Label	Storage
1	Blocking Reagent	Store at +15 to +25°C.

Storage Conditions (Working Solution)

Store the 10x stock solution of Blocking Reagent in aliquots at +2 to +8°C or –15 to –25°C.

1.3. Additional Equipment and Reagent required

For preparation of 10x Blocking Reagent stock solution and 1x Blocking solution

- i* See section, **Working Solution** for additional information on how to prepare solutions.
- Maleic acid buffer: maleic acid, NaCl, concentrated or solid NaOH
- i* Also available as *Maleic acid buffer, 10x, component of the DIG Wash and Block Buffer Set**.
- Heating block or microwave oven

For preparation of hybridization buffers

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

1.4. Application

The Blocking Reagent is used to decrease the background in nonradioactive hybridization and detection of nucleic acid hybrids.

2. How to Use this Product

2.1. Before you Begin

General Considerations

Hybridization 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.

Working Solution

Reagent/Buffer	Composition/Concentration
Maleic acid buffer	100 mM maleic acid, 150 mM NaCl, pH 7.5 (+20°C), adjusted with concentrated or solid NaOH, sterile.
10x Blocking Reagent stock solution	<ul style="list-style-type: none"> ▪ Dissolve Blocking Reagent 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. ▪ Autoclave stock solution prior to storage.
1x Blocking solution	Dilute the 10x Blocking Reagent stock solution with 1x Maleic acid buffer to a 1x-concentrated solution. ⚠ Always prepare fresh.

2.2. Protocols

Preparation of hybridization 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	<ul style="list-style-type: none"> ▪ 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	<ul style="list-style-type: none"> ▪ 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	<ul style="list-style-type: none"> ▪ 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.)

3. Supplementary Information

3.1. 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.

1 2 3 etc. Steps in a procedure that must be performed in the order listed.

* (Asterisk) The Asterisk denotes a product available from Roche Diagnostics.

3.2. Changes to previous version

Layout changes.

Editorial changes.

3.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
DIG Wash and Block Buffer Set	1 set, 30 blots (100 cm ²)	11 585 762 001
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
Sodium Dodecyl Sulfate (SDS)	1 kg	11 667 289 001
DIG Luminescent Detection Kit	1 kit, 50 blots of 10 cm x 10 cm	11 363 514 910
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 Nucleic Acid Detection Kit	1 kit, Detection of 40 blots of 10 cm x 10 cm	11 175 041 910
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-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
Buffers in a Box, Premixed SSC Buffer, 20x	4 l	11 666 681 001

3. Supplementary Information

3.4. Trademarks

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

3.5. License Disclaimer

For patent license limitations for individual products please refer to:

List of biochemical reagent products.

3.6. Regulatory Disclaimer

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

3.7. Safety Data Sheet

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

3.8. 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.

