Blocking Reagent

For nucleic acid hybridization and detection

Cat. No. 11 096 176 001

50 g

II Version 17

Stable at +15 to +25° C

Roche

Product overview

Appearence	Powder.
Application	The Blocking reagent is used to decrease the back- ground in non-radioactive hybridization and detection of nucleic acid hybrids.
Storage/ stability	The unopened reagent is stable at $+15$ to $+25^{\circ}$ C until the expiration date printed on the label.
	Note: We recommend to store the concentrated $10x$ Blocking solution in alignots at -15 to -25°C.

Preparation of 10x Blocking stock solution for filter hybridization

Additional required reagents	Maleic acid buffer: 100 mM Maleic acid, 150 mM NaCl, pH 7.5 (20°C), adjusted with conc. or solid NaOH, sterile.
Preparation of Blocking stock solution 10×	Blocking reagent is dissolved 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.
	This stock solution is autoclaved and stored at +2 to $+8^{\circ}$ C or -15 to -25°C subsequently.
Preparation of 1x Blocking solution	Dilute the 10x Blocking stock solution with 1x Maleic acid buffer to a 1x concentrated solution.
	Always prepare fresh!

Preparation of hybridization buffers

Use of Blocking	For the hybridization (and pre-hybridization) of filters
reagent in buffers	with DIG-labeled probes, the addition of Blocking
	reagent to the hybridization buffer is recommended.

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Hybridization buffer	Composition
Standard hybridiza- tion buffer	5x SSC; 0.1% N-lauroylsarco- sine (w/v), 0.02% SDS (w/v), 1 % Blocking solution (v/v) (1/10 volume of Blocking solu- tion, 10x conc.)
Standard hybridiza- tion buffer with forma- mide	50% Formamide (v/v) deion- ized, 5x SSC; 0.1% N-lauroyl- sarcosine (w/v), 0.02% SDS (w/v), 2% Blocking solution (v/v) (1/5 volume of Blocking solution, 10x conc.)
High SDS hybridiza- tion buffer	7% SDS, 50% formamide (v/v) deionized, 5x SSC, 50 mM sodium phosphate, pH 70, 0.1% N-lauroylsarcosine (v/v), 2% Blocking solution (w/v) (1/5 volume of Blocking solu- tion, 10× conc.)

Hybridization conditions

The hybridization conditions depend largely on the type of probe (DNA, RNA or oligonucleotide) and are described in detail in the working procedures of the corresponding DIG kits (see below).

Detailed working instructions and practical hints, concerning probe labeling with DIG , hybridization and chemiluminescent or color detection, are described in the DIG Kits (listed below) and in the DIG Applications Guide for Filter Hybridization.

Ordering Information

Kits		
Product	Pack Size	Cat. No
DIG High Prime Labeling and Detection Starter Kit I	12 labeling reactions and 24 blots	11 745 832 910
DIG High Prime Labeling and Detection Starter Kit II	12 labeling reactions and 24 blots	11 585 614 910
DIG Northern Starter Kit	10 labeling reactions and detection	12 039 672 910
DIG DNA Labeling and Detection Kit	25 labeling reactions and 50 blots	11 093 657 910
DIG Nucleic Acid Detection Kit	40 blots (10 x10 cm)	11 175 041 910
DIG Luminescent Detection Kit	50 blots (10 x10 cm)	11 363 514 910

Single reagents

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Product	Pack Size	Cat. No.	
DIG Easy Hyb	500 ml	11 603 558 001	
DIG Easy Hyb Granules	6x 100 ml	11 796 895 001	
Nylon membranes, positively charged	10 sheets (20x30 cm) 20 sheets (10x15 cm) 1 roll (0.3x3 m)	11 209 272 001 11 209 299 001 11 417 240 001	
Nylon membranes for Colony and Plaque Hybridization	50 discs (each 82 mm diameter) 50 discs (each 132 mm diameter)	11 699 075 001 11 699 083 001	
Blocking reagent	50 g	11 096 176 001	
Hybridization bags	50 bags	11 666 649 001	
Anti digoxigenin-AP conjugate , Fab fragments	150 U(200 μl)	11 093 274 910	
NBT/BCIP stock solution tablets	8 ml	11 681 451 001	
CSPD	1 ml	11 655 884 001	
CSPD, ready-to-use	2x 50 ml	11 755 633 001	
CDP-Star	1 ml	11 685 672 001	
CDP-Star, ready-to-use	2x 50 ml	12 041 677 001	
Tween 20	5x 10 ml	11 332 465 001	

* available from Roche Diagnostics.



Changes to previous version	Editorial changes.
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Disclaimer of License	For patent license limitations for individual products please refer to: <u>List of biochemical reagent products</u>

Contact and Support

To ask questions, solve problems, suggest enhancements and report new applications, please visit our $\underline{\textbf{Online Technical Support}}$ Site.

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



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