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

BM Purple

Version: 21
Content Version: November 2021

AP Substrate precipitating (NBT/BCIP ready-to-use)

Cat. No. 11 442 074 001 100 ml

Store the product at +2 to +8°C.

1. General Information

1.1. Contents

Vial / bottle	Label	Function / description	Content
1	BM Purple	Ready-to-use solution.	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	BM Purple	Store at +2 to +8°C. ⚠ ***Keep protected from light.***

1.3. Additional Equipment and Reagent required

For western blotting

- Phosphate buffered saline (PBS)*
- Tween 20* (v/v)
- Blocking Reagent* or
- PBS containing 1 to 5% bovine or horse serum (v/v) (optional)
- Antibody-AP conjugate*
- Petri dishes or shallow trays
- Rotating plate shaker
- Nitrocellulose membranes
- Filter paper
- Double-distilled water

1.4. Application

BM Purple can be used in the following applications:

- Immunohisto/cytochemistry
- Western blot
- Southern blot
- Northern blot
- Colony and plaque hybridization
- *In situ* hybridization

ⓘ *These assays may be either qualitative or quantitative.*

2. How to Use this Product

2.1. Before you Begin

General Considerations

Handling instructions

- Equilibrate the substrate to +15 to +25°C before staining and use it directly from the bottle. Do not dilute it.
- Wash blots in Petri dishes or shallow trays on a rotating plate shaker.
- To minimize use of reagents, prepare spot blots in 1 × 8 cm nitrocellulose strips and lay them in 10 cm Petri dishes. Carefully pipette 2 ml antibody conjugate directly onto each strip and incubate.
- Background staining should be negligible or, if present, should fade with drying. If it persists, try increasing the serum concentration in the Blocking buffer, increasing blocking time, diluting the conjugate, or testing a different batch of nitrocellulose.

Working Solution

Solution	Preparation/Composition	For use in...
PBST	Phosphate buffered saline (PBS), pH 7.2 to 7.5, containing 0.2% Tween 20* (v/v)	Washing
Blocking solution	Blocking Reagent* or PBS* containing 1 to 5% bovine or horse serum (v/v)	Membrane blocking

2.2. Protocols

Western blotting

See section, **Working Solution** *for additional information on preparing solutions.*

Perform membrane ELISA or western blotting on nitrocellulose according to standard protocols.

1. Apply antigen to membrane, 1 to 2 µg per spot.
2. Wash membrane twice in PBST.
3. Block remaining protein binding sites with Blocking reagent or horse serum.
4. Wash membrane twice in PBST.
5. Incubate with antibody-AP conjugate*.
6. Wash membrane twice with PBST.
7. Rinse with double-distilled water.
8. Equilibrate BM Purple substrate to +15 to +25°C; invert once to mix.
9. Incubate membrane in BM Purple substrate at +15 to +25°C.

   Color development may be finished in 30 minutes.

⑩ Rinse with double-distilled water.

⑪ Dry between sheets of filter paper or paper towel.

⑫ Store in the dark.

The colored reaction product is stable when dried.

In situ hybridization

⚠ ***For nonradioactive in situ hybridization with alkaline phosphatase and BM Purple substrate, it is important not to use xylene-based mounting media since these could lead to crystal formations of the color precipitates.***

3. Additional Information on this Product

3.1. Test Principle

Background information

BM Purple is a chromogenic substrate for alkaline phosphatase (AP) designed for precipitating enzyme immunoassays. It develops a permanent, dark purple band or spot at the AP binding site on the membrane or solid support. The stabilized substrate solution may be used directly from the bottle with no mixing or reconstitution. BM Purple produces negligible background staining for more sensitivity.

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		
Tween 20	50 ml, 5 x 10 ml	11 332 465 001
Blocking Reagent	50 g	11 096 176 001
Buffers in a Box, Premixed PBS Buffer, 10x	4 l	11 666 789 001
Anti-Digoxigenin-AP, Fab fragments	150 U, 200 µl	11 093 274 910
Anti-Fluorescein, Fab fragments	Anti-Fluorescein-AP, Fab fragments, 150 U, 200 µl	11 426 338 910
	Anti-Fluorescein-POD, Fab fragments, 150 U	11 426 346 910
Streptavidin Conjugates	Streptavidin-AP Conjugate, 1,000 U	11 089 161 001
	Streptavidin-POD Conjugate, 500 U	11 089 153 001

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

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