

BM Blue POD substrate, precipitating

Ready-to-use solution

Cat. No. 11 442 066 001

100 ml

Version 09
Content version: August 2018

Store at +2 to +8°C

Store protected from light!

Product overview

Product description BM blue is a chromogenic substrate for Peroxidase (POD) designed for precipitating enzyme immunoassays. It develops a permanent, dark blue band or spot at the POD binding site on the membrane or solid support. The stabilized substrate solution may be used directly from the bottle with no mixing or reconstitution. It is more sensitive than DAB and gives less background staining than Alkaline Phosphatase reactions.

Application BM blue is suitable *e.g.*, for

- precipitating enzyme immunoassays,
- Southern Blots
- Northern Blots
- Western Blots
- antibody-coated beads

These assays may be either qualitative or quantitative.

Storage/ Stability Stable, when stored protected from light, at +2 to +8°C until the expiration date printed on the label.

Protocol for Immunoblots

- Handling instructions**
- Bring 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 on 1 × 8 cm nitrocellulose strips and lay them in 10 cm petri dishes. Carefully pipette 2 ml antibody conjugate (working concentration ≤ 100 mU/ml) directly onto each strip and incubate.
 - Should the background staining start to take on a blue color, the reaction should be stopped immediately by aspirating the substrate and adding double dist. water. 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.
 - The substrate is very sensitive. If the colored band or spot is too heavy or flakes off, try diluting either the sample or the POD-conjugate.

- Additional reagents required**
- For washing purposes: PBST: Phosphate buffered saline (PBS), pH 7.2 – 7.5 containing Tween, 0.2% (v/v).
 - For the blocking of membranes: Blocking Reagent (*e.g.*, Blocking Reagent*).
Note: Bovine serum albumin is normally less suitable than Blocking Reagent.
 - Antibody-POD conjugate

Procedure

Carry out your membrane ELISA or immunoblot on nitrocellulose according to standard protocols.

Step	Action
1	Apply antigen to membrane, 1 – 2 µg per spot.
2	Wash membrane twice in PBST.
3	<ul style="list-style-type: none">▪ Saturate remaining protein binding sites with Blocking Reagent (0.5 – 1%).▪ Wash membrane twice.
4	<ul style="list-style-type: none">▪ Incubate with antibody-POD conjugate diluted with Blocking Reagent (0.5–1%).▪ Wash membrane twice.
5	Rinse with double dist. water.
6	Bring substrate to +15 to +25°C and invert once to mix.
7	Incubate membrane in substrate at +15 to +25°C. Note: Stop color development after 3 – 15 min, or at the latest when background staining appears, by aspirating the substrate and carefully (substrate bands may be damaged) adding double dist. water.
8	Rinse with double dist. water and dry on filter paper. Note: Store in the dark. The colored reaction product is stable when dried.

Ordering Information

POD-coupled Antibodies

Product	Pack size	Cat. No.
Anti-Digoxigenin-POD, Fab fragments from sheep	150 U	11 207 733 910
Anti-Digoxigenin-POD (poly), Fab fragments from sheep	50 U	11 633 716 001
Anti-Biotin-POD, Fab fragments (clone 33, mouse IgG1)	150 U	11 426 311 001
Anti-Fluorescein-POD, Fab fragments from sheep	150 U	11 426 346 910
Streptavidin-POD	500 U (1 ml)	11 089 153 001

Chromogene Substrates for Alkaline Phosphatases

Product	Pack size	Cat. No.
BCIP, crystals	200 mg	11 017 365 001
BCIP, 4-Toluidinsalz, powder	250 mg 1 g	10 760 994 001 11 585 002 001
BCIP, 4-toluidinesalt, solution	3 ml (150 mg)	11 383 221 001
INT/BCIP Stock Solution	3 ml	11 681 460 001
Fast Red Tablets	20 tablets	11 496 549 001
NBT/BCIP ready-to-use tablets	20 tablets	11 697 471 001
NBT/BCIP Stock Solution	8 ml	11 681 451 001
NBT Solution	3 ml (300 mg)	11 383 213 001
NBT crystals	5 g	11 585 029 001

Labeling of Biomolecules

Product	Pack size	Cat. No.
DIG RNA Labeling Mix	40 µl (20 reactions)	11 277 073 910
DIG DNA Labeling Mix	50 µl (25 reactions)	11 277 065 910
DIG High Prime	160 µl (40 labeling reactions)	11 585 606 910

Other reagents

Product	Pack size	Cat. No.
Tween 20, 10% (v/v)	5 × 10 ml	11 332 465 001
Blocking Reagent	50 g	11 096 176 001

* available from Roche Diagnostics.

Trademark	All third party product names and trademarks are the property of their respective owners.
Changes to previous version	Editorial changes.
Regulatory Disclaimer	For life science research only. Not for use in diagnostic procedures.
Disclaimer of License	For patent license limitations for individual products please refer to: List of biochemical reagent products

Contact and Support

To ask questions, solve problems, suggest enhancements and 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.



Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim
Germany