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

DAB Substrate

Version: 09
Content Version: December 2020

Metal enhanced, precipitating

Cat. No. 11 718 096 001 1 pack

Store the product at −15 to −25°C.

1. General Information

1.1. Contents

Vial / bottle	Label	Function / description	Content
1	DAB Substrate, DAB/metal concentrate, 10x conc.	Packaged under nitrogen for long-term stability.	1 bottle, 25 ml
2	DAB Substrate, Peroxide buffer	For preparation of working solution.	1 bottle, 250 ml

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	DAB/metal concentrate, 10x conc.	Store at −15 to −25°C. ⚠ ***Do not bring solution to +15 to +25°C; remove desired quantity and return bottle to −15 to −25°C.*** ⚠ ***For storage >6 months, replace nitrogen by gently bubbling a slow stream of nitrogen into the DAB/metal liquid.***
2	Peroxide buffer	Store at −15 to −25°C. ⓘ *For convenience, store at +2 to +8°C through the expiry date printed on the label.*

1.3. Additional Equipment and Reagent required

For immunohistocytochemistry

- 0.3% H_2O_2
- Methanol
- Wash buffer/PBS*
- Normal serum or Blocking buffer
- Humidified chamber
- Primary antibody
- POD-conjugated secondary antibody

For western blotting

- Membrane
- Blocking buffer
- Primary antibody
- Wash buffer/PBS*
- POD-conjugated secondary antibody

For *in situ* hybridization

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

- 50x FPG
- 20x SSC* buffer
- Hybridization buffer
- Blocking buffer
- TE buffer
- Wash buffer
- Biotin-labeled probe
- POD-conjugated streptavidin
- DNA, Sodium Salt from fish sperm*

1.4. Application

Use DAB Substrate for:

- Immunohistocytochemistry
- Immunoblotting
- *In situ* hybridization

Product Description

The metal enhanced DAB Substrate utilizes cobalt chloride and nickel chloride in a special formulation to produce a dark brown/black precipitate in the presence of horseradish peroxidase (POD).

2. How to Use this Product

2.1. Before you Begin

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

Solution	Preparation/Composition	For use in...
DAB Substrate working solution	▪ Determine the volume of substrate required for the development of POD. ▪ Remove the DAB/metal concentrate, 10x conc. (Bottle 1) from −15 to −25°C storage. ⚠ ***Do not bring to +15 to +25°C.*** ▪ Dilute the DAB/metal concentrate, 10x conc. with Peroxide buffer (Bottle 2) to a 1x working solution. – For example, if 5 ml of substrate are required, dilute 500 µl of the DAB/metal concentrate, 10x conc. with 4.5 ml of the Peroxide buffer. – Mix thoroughly. ▪ Add the 1x working solution to the tissue or blot until desired substrate development. ⓘ *The 1x solution is stable over many hours. For best results, store the working solution at +2 to +8°C when not using.*	▪ Immunohistocytochemistry ▪ Western blotting ▪ *In situ* hybridization
Blocking buffer	0.1 M Tris-HCl*, 0.1% BSA (w/v), 0.1% Tween 20* (v/v)	▪ Immunohistocytochemistry ▪ Western blotting ▪ *In situ* hybridization
Wash buffer	Phosphate buffered saline (PBS*)	▪ Immunohistocytochemistry ▪ Western blotting ▪ *In situ* hybridization
50x FPG	1% Ficoll 400 1% polyvinylpyrrolidone 360 1% glycine	*In situ* hybridization
20x SSC buffer	150 mM NaCl, 5 mM sodium citrate, pH 7.0	*In situ* hybridization
Hybridization buffer	50% deionized Formamide* 5x SSC* 1.25x FPG 31 mM KH_2PO_4 0.25% SDS* 31 µg/ml salmon sperm DNA* 5% dextran sulfate	*In situ* hybridization
TE buffer	10 mM Tris-HCl*, pH 7.6, 1 mM EDTA	*In situ* Hybridization

2.2. Protocols

Immunohistocytochemistry

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

1. Prepare sample material, such as tissue sections and cell preparations.

2. Block the endogenous peroxidase activity by using 0.3% H_2O_2 in methanol for 30 minutes at +15 to +25°C.

3. Rinse slides in Wash buffer.
   – Block nonspecific binding sites in the sample material with normal serum or other Blocking buffer for 30 minutes at +15 to +25°C in a humidified chamber.
   – Pour off the Blocking buffer but do not remove excess.

4. Incubate the sample material with the primary antibody for 30 minutes at +15 to +25°C in a humidified chamber.

5. Wash the sample material in buffer, such as Wash buffer for 3 minutes.
   – Repeat washing step.
   ⚠ ***Remove excess Wash buffer.***

6. Incubate the sample material with POD-conjugated secondary antibody for 30 minutes at +15 to +25°C in a humidified chamber.

7. Rinse slides in Wash buffer for 3 minutes.
   – Repeat washing step.
   ⚠ ***Remove excess Wash buffer.***

8. Add the DAB Substrate working solution and incubate until desired staining has been achieved.

   Typical incubations are from 5 to 15 minutes.

Western blotting

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

1. Transfer protein from the gel to a membrane.

2. Remove membrane and block the nonspecific binding sites with a Blocking buffer for 10 to 30 minutes at +15 to +25°C with shaking.

3. Incubate the blot with the primary antibody for 1 hour at +15 to +25°C with shaking.

4. Wash the membrane with Wash buffer.

5. Incubate the blot with POD-conjugated secondary antibody for 1 hour at +15 to + 25°C with shaking.

6. Wash the membrane with Wash buffer.

7 Add DAB Substrate working solution as shown in the table:

If you use the...	Then add...
tank technique	100 – 125 µl/cm
transparency technique	10 – 20 µl/cm

DAB substrate working solution should be added until desired development is achieved. Typical incubations are from 5 to 15 minutes.

In situ hybridization

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

1 Prepare tissue.

2 Pretreat slides with 1x FPG for 3 hours at +65°C to block nonspecific binding.

3 Prehybridize slides with Hybridization buffer without probe for 60 minutes.

4 Add Hybridization buffer containing 100 to 500 ng/ml biotin-labeled probe.

5 Detect targets according to the following table:

Detection of...	Then...
DNA targets	Incubate the slide at +90°C for 4 minutes, followed by a quick cooling in ice for 4 minutes to denature probe and target DNA.
RNA targets	Denature the probe DNA prior to applying to the slide.

6 Hybridize overnight at +45°C.

7 Wash slides with the following solutions with gentle agitation:
- 2x SSC for 60 minutes.
- 1x SSC for 60 minutes.
- 0.2x SSC for 30 minutes.

8 Block the sample with a Blocking buffer for 30 to 60 minutes at +15 to +25°C.

9 Add POD-conjugated streptavidin and incubate for 1 hour at +15 to +25°C.

10 Wash sample with Wash buffer.

11 Add DAB Substrate working solution to the sample and develop signal.

12 Stop development by rinsing slides in TE buffer.

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.
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.

3.2. Changes to previous version

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

3.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
Formamide	500 ml	11 814 320 001
DNA	custom fill	10 223 638 103
Tris hydrochloride	500 g	10 812 846 001
Tween 20	50 ml, 5 x 10 ml	11 332 465 001
Buffers in a Box, Premixed PBS Buffer, 10x	4 l	11 666 789 001
Buffers in a Box, Premixed SSC Buffer, 20x	4 l	11 666 681 001
Sodium Dodecyl Sulfate (SDS)	1 kg	11 667 289 001

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.

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