Metal Enhanced DAB Substrate Kit

Catalog Number 34065

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WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

Product description

The Thermo Scientific™ Metal Enhanced DAB Substrate Kit contains a special formulation of cobalt chloride and nickel chloride to produce a dark brown/black precipitate in the presence of horseradish peroxidase (HRP) in immunohistochemical, immunoblotting and *in situ* hybridization applications.

Contents and storage

Table 1 Metal Enhanced DAB Substrate Kit

Material	Amount	Storage [1]
DAB/Metal Concentrate (10X)	25 mL	–20°C
Stable Peroxide Buffer	250 mL	4°C

[1] Product is shipped with dry ice

Required materials not supplied

Item	Source
Thermo Scientific™ SuperBlock Blocking Buffer in TBS	37535
Thermo Scientific™ SuperBlock Blocking Buffer in PBS	37515
Thermo Scientific™ Peroxidase Suppressor	35000

Procedural guidelines

- Store the DAB/Metal Concentrate (10X) at or below –20°C.
- When using the solution, take out the necessary quantity and promptly put the bottle back at –20°C.
- Do not allow the solution to equilibrate to room temperature.
- The solution is packaged with nitrogen to ensure long-term stability.
- After each use, replace the nitrogen by gently bubbling a slow stream of nitrogen into the solution.
- The Stable Peroxide Buffer contains the optimal concentration of hydrogen peroxide.

  Note: Do not add hydrogen peroxide to the Stable Peroxide Buffer, doing so will increase background intensity.
- The Stable Peroxide Buffer activity is not affected by storage at –20°C or by freezing/thawing.
- Precipitation may occur but does not negatively affect product performance.

Prepare Metal Enhanced DAB Substrate working solution

1. Remove the DAB/Metal Concentrate (10X) from –20°C storage.
2. Mix the concentrate well by inverting the bottle.
3. Remove the required quantity for use and return the bottle to –20°C storage.
4. Prepare a 1X working solution of the DAB/Metal Concentrate (10X) by adding the Stable Peroxide Buffer and mixing well.

   Note: For example, to make 5 mL of substrate add 4.5 mL of the Stable Peroxide Buffer to 500 µL of the DAB/Metal Concentrate. The 1X substrate solution remains stable for several hours at 4°C.
5. Proceed to IHC staining or western blot detection.

Typical example of Immunohistochemical (IHC) staining

Below steps indicate an example procedure for immunohistochemical staining:

1. Block nonspecific sites of prepared tissue using normal serum or another blocking solution, SuperBlock Blocking Buffer in TBS (Catalog No. 37535) or SuperBlock Blocking Buffer in PBS (Catalog No. 37515), for 30 minutes at room temperature in a humidity chamber. Decant the blocking buffer but leave any excess in place.
2. Incubate tissue with the primary antibody for 30 minutes at room temperature in a humidity chamber.
3. Wash tissue in buffer (e.g., TBS or PBS) for 3 minutes. Repeat wash step. Remove excess wash buffer.
4. Suppress endogenous peroxidase activity using Peroxidase Suppressor (Catalog No. 35000). Incubate for 15–30 minutes at room temperature in a humidity chamber.
5. Repeat step 3
6. Incubate tissue with HRP-conjugated secondary antibody for 30 minutes at room temperature in a humidity chamber.

   Note: The ABC (Avidin-Biotin Complex) System can also be used. See **thermofisher.com** for available ABC kits.
7. Repeat step 3
8. Add the Metal Enhanced DAB Substrate working solution and incubate until the desired staining is achieved. Typical incubations are from 5–15 minutes.

Typical example of Western blot (WB) detection

Below steps indicate an example procedure for western blot detection:

1. Transfer protein from the gel to a membrane.
2. Remove membrane and block the nonspecific sites on the membrane with a blocking buffer, SuperBlock Blocking Buffer in TBS (Catalog No. 37535) or SuperBlock Blocking Buffer in PBS (Catalog No. 37515), for 10–30 minutes with constant shaking.
3. Add primary antibody and incubate membrane for 1 hour with shaking.
4. Wash the membrane with wash buffer (e.g., TBS or PBS).
5. Add the HRP-conjugated secondary antibody and incubate membrane for 1 hour at room temperature with shaking.
6. Incubate tissue with HRP-conjugated secondary antibody for 30 minutes in a humidity chamber.
7. Repeat step 4
8. Add Metal Enhanced DAB Substrate working solution and incubate membrane until the desired development is achieved. Typical incubations are from 5–15 minutes.

Troubleshooting

Observation	Possible cause	Recommended action
Precipitate is brown instead of black/brown	Cobalt and nickel are heavy metals and will separate during storage.	Mix by inverting the bottle before use to obtain a homogeneous solution of DAB and metals.
Background is dark, reducing the signal-to-noise ratio	DAB/Metal Concentrate was left at room temperature.	Store the DAB/Metal Concentrate at or below –20°C to prevent excess background.
High background	Too much HRP in the system.	Use less antibody in the system as this substrate is 50 times more sensitive than DAB without metals and requires much less antibody for detection.

Documentation and support

Customer and technical support

Visit **thermofisher.com/support** for the latest service and support information.

- Worldwide contact telephone numbers
- Product support information
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- Product documentation
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

  Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and its affiliates warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have questions, contact Life Technologies at **www.thermofisher.com/support**.

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: Pub. No. MAN0011292 B

Revision	Date	Description
B	28 October 2024	New document created for Metal Enhanced DAB Substrate Kit in CCMS. The document was updated to the current template, with associated updates to the warranty, trademarks, and logos.
A	17 October 2015	Baseline for this revision history.