INSTRUCTIONS

Metal Enhanced DAB Substrate Kit

34065

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Number	Description
34065	**Metal Enhanced DAB Substrate Kit**
	Kit Contents:
	DAB/Metal Concentrate (10X), 25mL
	Stable Peroxide Buffer, 250mL
	Storage: Upon receipt store the DAB/Metal Concentrate at -20°C and the Stable Peroxide Buffer at 4°C. Product is shipped with dry ice.

Introduction

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.

Important Product Information

- Store the DAB/Metal Concentrate at or below -20°C. To use the solution, remove the quantity required for use and immediately return the bottle to -20°C. Do not allow the solution to equilibrate to room temperature. The solution is packaged under nitrogen for long-term stability; after every 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. Do not add hydrogen peroxide to the Stable Peroxide Buffer, which will increase background. The Stable Peroxide Buffer activity is not affected by storage at -20°C or by freezing/thawing. For convenience, store the product at 4°C to eliminate thawing.

Preparation of Metal Enhanced DAB Substrate Working Solution

Please read all instructions before beginning the procedure.

1. Remove the DAB/Metal Concentrate (10X) from -20°C storage and mix well by inverting the bottle. Remove quantity required for use and immediately return bottle to -20°C.
2. Prepare a 1X working solution of the DAB/Metal Concentrate (10X) by adding the Stable Peroxide Buffer and mixing well. For example, if 5mL of substrate is required, add 4.5mL of the Stable Peroxide Buffer to 500µL of the DAB/Metal Concentrate. The 1X substrate solution is stable for several hours at 4°C.

Example Procedure for Immunohistochemical Staining

1. After tissue has been prepared, block nonspecific sites with normal serum or other blocking solution such as Thermo Scientific SuperBlock Blocking Buffer in TBS or PBS (Product No. 37535 or 37515) for 30 minutes at room temperature in a humidity chamber. Decant blocking buffer but do not remove excess.
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 the endogenous peroxidase activity using Thermo Scientific Peroxidase Suppressor (Product No. 35000). Incubate for 15-30 minutes at room temperature in a humidity chamber.
5. Wash tissue in buffer for 3 minutes. Repeat wash step. Remove excess wash buffer.
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 may also be used. See the catalog for available ABC kits.

Pierce Biotechnology 3747 N. Meridian Road | PO Box 117 Rockford, IL 61105 USA | (815) 968-0747 (815) 968-7316 fax | www.thermoscientific.com/pierce

7. Wash tissue in buffer for 3 minutes. Repeat wash step. Remove excess wash buffer.
8. Add the Metal Enhanced DAB Substrate Working Solution and incubate until the desired staining is achieved. Typical incubations are from 5 to 15 minutes.

Example Procedure for Western Blot Detection

1. Transfer protein from the gel to a membrane.
2. Remove membrane and block the nonspecific sites with a blocking buffer such as SuperBlock® Blocking Buffer in TBS or PBS (Product No. 37535 or 37515) for 10-30 minutes at room temperature with shaking.
3. Add the 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. Wash membrane with wash buffer.
7. Add the Metal Enhanced DAB Substrate Working Solution and incubate membrane until the desired development is achieved. Typical incubations are from 5 to 15 minutes.

Troubleshooting

Problem	Possible Cause	Solution
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
The DAB/Metal Concentrate contains a precipitate in the bottle	DAB/Metal Concentrate was not maintained at -20°C	Do not use substrate that contains a precipitate in the bottle
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

Product References

Chan, J.R., *et al.* (2000). Progesterone synthesized by schwann cells during myelin formation regulates neuronal gene expression. *Mol Biol Cell* **11:**2283-95.

Itoh, H. (2000). Upregulation of HGF activator inhibitor type 1 but not type 2 along with regeneration of intestinal mucosa. *Am J Physiol Gastrointest Liver Physiol* **278:**635-43.

Kaushic, C., *et al*. (2000). Effects of estradiol and progesterone on susceptibility and early imune responses to *Chlamydia trachomatis* infection in the female reproductive tract. *Infec Immunity* **7:**4207-16.

Martin, M.N. and Slovin, J.P. (2000). Purified γ-glutamyl transpeptidases from tomato exhbit high affinity for glutathione and glutathione S-conjugates. *Plant Physiol* **122:**1417-26.

Scidmore, M.A., *et al.* (2003). Restricted fusion of *Chlamydia trachomatis* vesicles with endocytic compartments during the initial stages of infection. *Infec Immunity* **2:**973-84.

Current product instructions are available at *www.thermoscientific.com/pierce*. For a faxed copy, call 800-874-3723 or contact your local distributor.