Product Information

SIGMA*FAST*™ 3,3′-Diaminobenzidine tablets

Tablet, to prepare 1 mL

D4168

Product Description

Diaminobenzidine (DAB) is used in many applications for visualization of peroxidase activity.[1-6] DAB is the immunohistology substrate of choice, as it produces an intense brown-black stain, which is resistant to alcohol. Slides stained with DAB may be cover-slipped in the traditional manner and stored for future reference.

SIGMA*FAST*™ 3,3′-Diaminobenzidine tablets have been developed for use in immunohistology as a precipitating substrate to detect peroxidase activity:

Peroxidase + 2 $H_2O_2 \rightarrow O_2$ + 2 H_2O (pH 7.6)

O_2 + DAB → insoluble, brown-black precipitate

SIGMA*FAST*™ DAB tablets require no additional ingredients or procedures to prepare an active substrate solution. One SIGMA*FAST*™ DAB tablet set (one DAB tablet and one Urea Hydrogen Peroxide tablet) dissolved in 1 mL of ultrapure water provides 1 mL of a ready-to-use substrate solution that contains:

- 3,3′-Diaminobenzidine (DAB): 0.7 mg/mL
- Urea Hydrogen Peroxide (H_2O_2 equivalence, 0.7 mg/mL): 2.0 mg/mL
- Trizma® buffer: 60 mM

This product has been used to study such systems as fish liver,[7] rat thymus,[8] simian virus-transformed cell lines,[9] frontal cortex,[10] mouse lung,[11] and human decidual and placental tissues.[12] Several theses[13,14] and dissertations[16-32] have cited use of product D4168 in their research protocols.

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

Store the tablets at –20 °C.

Components

- 3,3′-Diaminobenzidine (DAB) tablets (Component Number D9167): 5SET (5 tablets) or 50SET (50 tablets)
- Urea Hydrogen Peroxide tablets (Component Number U5005): 5SET (5 tablets) or 50SET (50 tablets)

Reagents and Equipment Required but Not Provided

- Ultrapure water (17 MΩ•cm or equivalent)
- Pipette capable of delivering 5 mL
- Test tubes

Additional Optional Materials

- 0.2 µm filter (such as Cat. No. WHA10462701)
- Nickel(II) chloride ($NiCl_2$) or Cobalt(II) chloride ($CoCl_2$), to prepare 0.3% (w/v) stock solution to enhance tissue stains
- PBS for washing

Preparation Instructions

1. Remove the required number of DAB and Urea Hydrogen Peroxide tablets from the freezer.
2. Allow the tablets to reach room temperature.
3. Open DAB tablet package (silver foil) and Urea Hydrogen Peroxide tablet package (gold foil). Drop the tablets into an appropriate container. **Do not touch the tablets with your fingers**.
4. Add 5 mL of ultrapure water.
5. Vortex until dissolved.

The SIGMA*FAST*™ DAB Substrate Solution is now ready for use. For best results, the solution should be used within one hour.

Procedure

1. Cover the treated tissue section with 0.2-0.5 mL of DAB Substrate Solution.
2. The DAB reaction may occur rapidly. Color development should be carefully monitored during the reaction to prevent overdevelopment and high backgrounds. Reactions may be stopped by gently washing the slide in water or PBS.
3. DAB reactions may be enhanced by the addition of a $NiCl_2$ or $CoCl_2$ solution. Add 0.1 mL of 0.3% (w/v) stock solution to 0.9 mL DAB Substrate Solution. The addition of metal salts to DAB changes the color of the precipitate product to black or blue-black.
4. Occasionally the DAB Substrate Solution may be hazy. The haziness may be removed by filtering the solution through a 0.2 µm filter.
5. Tissues stained with SIGMA*FAST*™ DAB Substrate Solution may be dehydrated with alcohol and mounted with traditional resinous mounting media.

Troubleshooting

Background is too high

1. Use a blocking step prior to the application of the primary antibody. Diluted normal serum (10% v/v) from the same species as the secondary antibody generally produces the best results.
2. Block endogenous peroxidase by flooding the slide with a solution of 4 parts methanol and 1 part 3% H_2O_2 solution.
3. Decrease the staining time.
4. Titer the conjugate to optimize working dilution.

No color develops or color is too faint

1. Adjust the concentration of the primary antibody.
2. Adjust the concentration of the secondary antibody.
3. Determine if the enzyme conjugate is active.
4. Consider using an amplifying system such as avidin-biotin or peroxidase anti-peroxidase.
5. Increase the staining time.
6. Determine if enzymatic treatment (unmasking) of the antigen is required prior to application of the primary antibody.

References

1. Nakane, P.K., and Pierce, G.B., Jr., *J. Histochem. Cytochem.*, **14(12)**, 929-931 (1966).
2. Trojanowski, J.Q. *et al*., *J. Histochem. Cytochem.*, **31,** 1217-1223 (1983).
3. DeJong, A.S.H. *et al*., *Histochem. J*., **17(10)**, 1119-1130 (1985).
4. Chu, N.M. *et al*., *J. Histochem. Cytochem*., **37(2)**, 257-263 (1989).
5. Merchenthaler, I. *et al*., "Silver Intensification in Immunocytochemistry", in *Techniques in Immunocytochemistry* (Bullock, G., and Petrusz, P., eds.). Academic Press Ltd. (San Diego, CA), pp. 217-252 (1989).
6. Hsu, S., and Soban, E., *J. Histochem. Cytochem*., **30(10)**, 1079-1082 (1982).
7. Cooper, P.S. *et al*., "Immunohistochemical and immunoblot detection of P-glycoprotein in normal and neoplastic fish liver", in *Techniques in Aquatic Toxicology*, Volume 1 (G.K. Ostrander, ed.). CRC Lewis Publishers, Chapter 17, pp. 307-325 (1996).
8. Funk, J.L. *et al*., *J. Anat.*, **194(Pt 2)**, 255-264 (1999).
9. Montano, X., and Rundell, K., *Methods Mol. Biol.*, **165**, 229-242 (2001).
10. Navarro, A. *et al.*, *Age (Dordr.)*, **32(1)**, 85-96 (2010).
11. Haegens, A. *et al*., *J. Immunol.*, **182(12)**, 7990-7996 (2009).
12. Vento-Tormo, R. *et al*., *Nature*, **563**, 347-353 (2018).
13. Black, Alicia, "Integrin alpha 1 beta 1 has limited influence on epidermal growth factor receptor signaling but sex-dependent effects on estrogen receptor beta in murine knee chondrocytes". University of Guelph, M.Sc. thesis, p. 55 (2020).
14. Pham, Diana Minh, "Modulation of TGFβ1-induced Fibroblast-to-Myofibroblast Transition in response to Prostaglandin E_2 Production by Human Rhinovirus-Infected Airway Epithelial Cells". University of Calgary, M.Sci. thesis, p. 38 (2020).
15. Cooper, Peter S., "Expression of the xenobiotic transporter P-glycoprotein in the mummichog (*Fundulus heteroclitus*)". College of William and Mary, Ph.D. dissertation, p. 34 (1996).
16. Zhang, Zuwen, "Functional analysis of the mouse *CBP* gene in the adult central nervous system". The Open University, Ph.D. dissertation, p. 65 (2002).
17. Bunaciu, Rodica Petruta, "The effect of polychlorinated biphenyls on liver tumor promotion: a role for Kupffer cells?" University of Kentucky, Ph.D. dissertation, p. 37 (2005).
18. Schock, Henning Wolfgang, "Expression von Sex Hormone-Binding Globulin und Androgenrezeptoren in Myokardbiopsien von Patienten mit dilatativer Kardiomyopathie" ("Expression of sex hormone-binding globulin and androgen receptors in myocardial biopsies from patients with dilated cardiomyopathy"). Friedrich-Schiller-Universität Jena, Dr. med. dissertation, p. 23 (2004).

19. Schupp, Kathrin, "*In vitro* Herstellung eines vorderen Kreuzbandkonstruktes aus mesenchymalen Stammzellen und einem Kollagen Typ I-Hydrogel" ("*In vitro* production of an anterior cruciate ligament construct mesenchymal stem cells and a collagen type I hydrogel"). Bayerischen Julius-Maximilians-Universität zu Würzburg, Dr. med. dissertation, p. 64 (2006).
20. Schulte, Dominik Maria, "Expression und spatio-temporale Verteilung von Differenzierungs- und Proliferationsfaktoren während der murinen Nebennierenentwicklung" ("Expression and spatio-temporal distribution of differentiation and proliferation factors during murine adrenal development"). Albert-Ludwigs-Universität Freiburg im Breisgau, Dr. med. dissertation, p. 15 (2007).
21. Rivkin, Elena, "Identification and analysis of the novel *gumby* gene and its vertebrate specific roles in the mouse". University of Toronto, Ph.D. dissertation, p. 65 (2010).
22. Weber, Peter, "RNA interference as a tool for functional neurogenetics and the role of microRNAs in brain function". Technische Universität München, Dr. rer. nat. dissertation, p. 69 (2010).
23. Fonken, Laura K., "Physiological Consequences of Circadian Disruption by Nighttime Light Exposure". The Ohio State University, Ph.D. dissertation, p. 72 (2013).
24. Chang, Zhe, "The mechanism of tumorigenesis in the immortalized human pancreatic cell lines: cell culture models of human pancreatic cancer". University of Texas Health Science Center at Houston, Ph.D. dissertation, p. 27 (2011).
25. Lieberman, Rebecca A., "The Role of Hypothalamic Inflammation in Dim Light at Night and High Fat Diet Induced Metabolic Disturbance". The Ohio State University, Ph.D. dissertation, p. 9 (2013).
26. Lean, Samantha C., "Advanced Maternal Age: Identifying Mechanisms Underlying Vulnerability to Stillbirth". University of Manchester, Ph.D. dissertation, p. 103 (2015).
27. Eftychidis, Vasileios, "Elucidating the principal role of cholecystokinin neurons of the ventromedial hypothalamic nucleus in energy homeostasis". University of Oxford, Ph.D. dissertation, p. 62 (2017).
28. Bisiak, Francesco, "Structural studies of the Roundabout protein family". Communauté Université Grenoble Alpes, Ph.D. dissertation, p. 27 (2018).
29. Ruivo, Jorge Manuel Arsénio dos Santos, "The use of miR-92a inhibitor to enhance endothelial progenitor cell-mediated regeneration of injured arteries". University College London, Ph.D. dissertation, p. 105 (2018).
30. Kaltenecker, Péter, "Investigating the role of Wnt/PCP proteins in axon growth and neuronal cytoskeleton regulation". University of Liverpool, Ph.D. dissertation, p. 61 (2019).
31. Byers, Kaleb Miles, "Platforms and molecular mechanisms for improving signal transduction and signal enhancement in multi-step point-of-care diagnostics". Purdue University, Ph.D. dissertation, p. 72 (2021).
32. Sewell, Yvonne Claire, "Modelling Human Glomerulosclerosis *in vitro*". University of Cambridge, Ph.D. dissertation, p. 44 (2021).

Notice

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

The information in this document is subject to change without notice and should not be construed as a commitment by the manufacturing or selling entity, or an affiliate. We assume no responsibility for any errors that may appear in this document.

Technical Assistance

Visit the tech service page at SigmaAldrich.com/techservice.

Standard Warranty

The applicable warranty for the products listed in this publication may be found at SigmaAldrich.com/terms.

Contact Information

For the location of the office nearest you, go to SigmaAldrich.com/offices.

D4168dat Rev 03/22 PCS,RBG,GCY,MAM