PolyFacts BioSciences

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News | Views | Insights *from* Polysciences, Inc. Chemistry beyond the ordinary

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CellVue® Fluorescent Dye Kits for Cell Membrane Labeling

What are they?

CellVue® dyes are sensitive probes for detecting rapid uniform membrane changes in any cell or bioparticle with a membrane. Suitable for cell tracking and proliferation studies and compatible with flow cytometers, confocal and *in vivo* imaging equipment. CellVue® labeled cells are brightly fluorescent, emitting in the long wavelength UV, the far red or the near infrared region of the spectrum and display potential shifts in their excitation and emission. This shift makes it possible to provide sensitive, quantitative methods for monitoring the fate of labeled cells at both macro and micro levels.

What do CellVue® Kits for Cell Membrane Labeling Offer?

- Versatility – use with any cell type or bioparticle with a membrane
- Provides stable labeling with minimal transfer from cell to cell
- Provides rapid, uniform membrane labeling
- Combine with fluorescent antibodies or markers of cell function
- Suitable for cell tracking and proliferation studies
- Several colors (UV to NIR) for multi-parameter studies (use with existing fluorochromes for more colors)
- Far-Red and NIR versions can provide greater signal to noise due to reduced background autofluorescence
- Compatible with flow cytometers, confocal and *in vivo* imaging equipment
- Convenient, easy-to-use kit format

How do they work?

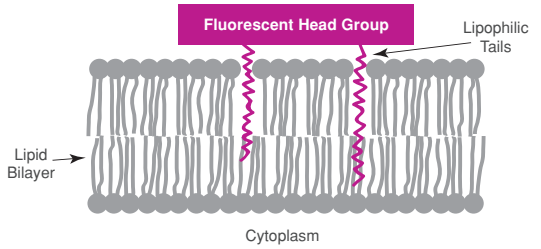
The CellVue® cell linker kits use proprietary membrane labeling technology to stably incorporate a fluorescent dye with long aliphatic tails into the lipid regions of the cell membrane,[1] see *Figure 1*. The labeling vehicle provided with the kit (Diluent C) is an iso-osmotic aqueous solution which contains no physiologic salts or buffers, detergents, or organic solvents and is designed to maintain cell viability while maximizing dye solubility and staining efficiency. The pattern of staining is dependent upon the cell type being labeled and the membranes of the cells.[2,3]

continues - page 2

What are the advantages of Far Red and Near Infrared Fluorescence?

- Reduced autofluorescence background
- Greater signal to noise
- Greater ability to multiplex with visible probes due to minimal spectral overlap
- Excellent for use in combination with other cell tracking probes such as CFSE or PKH26

Cell Tracking with Lipophilic Membrane Intercalating Dyes



Non-covalent labeling mediated by hydrophobic interactions between dye and membrane lipids

Figure 1

CellVue® Kits For Cell Membrane Labeling from pg. 1

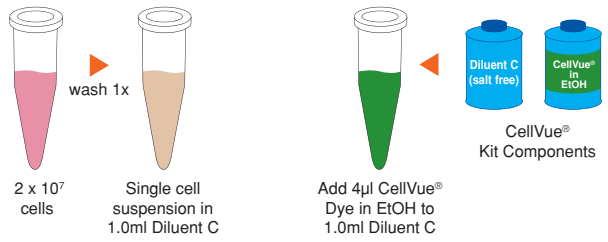
Methods for Cell Labeling with CellVue® Dyes

Mixing Steps and Critical Preparation Points

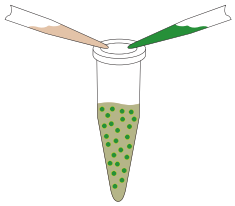
Simple Step by Step Mixing for CellVue® Dyes

A. Prepare Cells

B. Combine CellVue® Kit Components



C. Add equal volumes of 2X Cells and 2X Dye rapidly and thoroughly



D. Incubate 2-5 min. at 20-25°C

E. Add equal vol. FCS (fetal calf serum) or other protein to stop dye uptake

F. Wash 3x in medium +10% FCS; check recovery (>90%) and viability (>95%)

G. Resuspend in RPMI medium + 7% DMSO + 15% FCS; freeze at controlled rate; store at -80°C or in liquid nitrogen

Critical Points in Preparing Cells

- Use "happy" cells
- Aspirate carefully to minimize cell loss
- Disperse cells thoroughly
- Reproduce cell number and final volume precisely

Critical Points in Combining CellVue® Components Together

- Thoroughly disperse CellVue® Dye in Diluent C
- Minimize time between dye dilution and mixing with cells
- Reproduce volumes of CellVue® Dye and Diluent precisely

CellVue® Kits are available in 2 sizes:

Mini Kit *(small)*:

1 vial containing: 0.1ml, 1 X 10^{-3}M in ethanol fluorochrome dye stock and 1 vial containing 10ml of diluent.

Midi Kits *(medium)*:

2 vials containing: 0.1ml, 1 X 10^{-3}M in ethanol fluorochrome dye stock and 6 vials containing 10ml of diluent.

Technical Data Sheet Online
www.polysciences.com/shop/assets/datasheets/769.pdf

What are cell membrane labeling dyes used for?

Anticipated Applications of CellVue® Dye Labeling by Cell Type

Cell Type	Applications
Leukocytes	*in vivo* trafficking; TIL & LAK migration; cell conjugation; cytotoxic T cells/tumors; virus-infected cell migration; rheumatoid arthritis; lung macrophages, scid mouse transplants; rabbit granulocytes; mouse spleenocytes, mononuclear cells, differentiation; cell growth, cell adhesion; adoptive transfer; cytotoxicity; HIV binding; accountability; migration; bone marrow; lymph nodes.
Red Blood Cells	Survival, video imaging, malaria
Plant Cells	Protoplast fusion; parasite invasion
Bacteria, Fungi	Phagocytosis; proliferation/fermentation; fungal cell studies
Cell Lines, cultures	Isolating fast/slow growing clones; tumor spheroids; plasma membrane trafficking; tumor/macrophage interactions; hybridomas; endothelial cell attachment; proliferation rate
Marine Organisms	Sponge cells; sea urchin sperm; sea urchin embryos; algae; phytoplankton
Other Uses	Liposome targeting; neural cells; fibroblasts; epithelial cells; glial cells; glioma cells; amoebae; drug effects on cells; viral particle labeling; protozoa; keratinocytes; osteoblasts; chondrocytes

References

1. Horan, P. K., and Slezak, S. E., Nature, 340, 167168 (1989).
2. Horan, P.K., et al., Methods Cell Biol., 33, 469-490 (1990).
3. Poon, R.Y., et al., in: In Living Color: Flow Cytometry and Cell Sorting Protocols, Diamond, R. A., and DeMaggio, S. (Eds.). p. 302-352 (Springer-Verlag, New York, 2000).

Cat. #	Description		Ex. Max	Em. Max
24850	CellVue® Burgundy	**Midi Kit**	683nm	707nm
24843	CellVue® Burgundy	Mini Kit		
24849	CellVue® Claret	**Midi Kit**	655nm	675nm
24844	CellVue® Claret	Mini Kit		
24851	CellVue® Lavender	**Midi Kit**	425nm	461nm
24841	CellVue® Lavender	Mini Kit		
24847	CellVue® Maroon	**Midi Kit**	647nm	667nm
24840	CellVue® Maroon	Mini Kit		
24852	CellVue® NIR780	**Midi Kit**	743nm	776nm
24845	CellVue® NIR780	Mini Kit		
24853	CellVue® NIR815	**Midi Kit**	786nm	814nm
24846	CellVue® NIR815	Mini Kit		
24848	CellVue® Plum	**Midi Kit**	652nm	671nm
24842	CellVue® Plum	Mini Kit		

The Hope Revolution: HOPE® Fixation System

Stabilizes Cell Morphology and Antigen Expression

Questions and answers pertaining to Polysciences' HOPE® Fixation technique which allows greater morphology and retrospective studies on the molecular level.

Q. What is HOPE® Fixation System I and System II?

A. HOPE® Fixation is used in the preparation of paraffin embedded tissue sections allowing clearly visible molecular level results. In contrast to other fixation methods, HOPE® does not completely denature or crosslink structural proteins, enzymes or nucleic acids. They remain in an almost native state. Care must be taken when preparing this tissue which may include active prions, viruses or microorganisms. Universal precautions should be used when handling all specimens in the laboratory including wearing protective gloves.

Figure 1

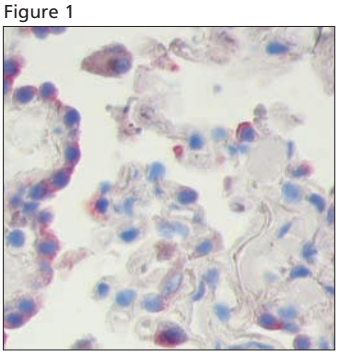


Figure 2

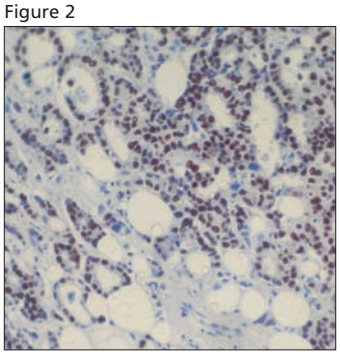


Figure 1: Immunohistochemistry
HOPE® Fixation allows for the detection of TLR2a in human lung via IHC using an antibody which is not recommended or intended for formalin fixed paraffin embedded tissue.

Figure 2: Immunohistochemistry without Additional Antigen Retrieval Steps
HOPE® fixed tissue used in the detection of estrogen receptor in human breast carcinoma via IHC without time consuming antigen retrieval steps.

Description	Cat. #	Size
HOPE® Fixative - System I	24823-500	500ml
HOPE® Fixative - System I	24823-2500	2500ml
HOPE® Fixative - System II	24824-1	1ml

Q. What applications can be performed using HOPE® fixed tissue?

A. Immunohistochemistry, *in situ* hybridization targeting DNA/RNA analysis, PCR, RT-PCR, Laser Capture Microdissection, FISH, Western Blot, Northern Blot and RNA microarray analysis.

Q. Do I need pre-treatment or HIER when using paraffin embedded blocks fixed with the HOPE® Fixation Technique?

A. No. Save up to 1 hour by using HOPE® Fixative because antigen retrieval steps are not necessary.

Q. Can antibodies be used that previously may not work on formalin fixed paraffin embedded tissue?

A. YES!

Q. What are the advantages to using the HOPE® Fixation technique?

A.
- Extract more DNA and RNA from HOPE® fixed paraffin embedded tissues than ever before!
- Save Time - Perform IHC on paraffin embedded tissue without further antigen retrieval steps, saving 20 min. to 1 hr.
- Compatibility - Allows the use of antibodies that are usually not recommended or intended for formalin fixed paraffin embedded tissue.
- Problem Free - No more overfixed formalin tissue samples.
- Outstanding Performance - Unmatched results in RNA and DNA in situ hybridization.
- Effective - Excellent conservation of nucleic acids for PCR and RT-PCR.

Technical Data Sheet Online
www.polysciences.com/shop/assets/datasheets/767.pdf

Photos Courtesy of Dr. T. Goldmann and Prof. Dr. E. Vollmer, Research Center Borstel and DCS – Innovative Diagnostik-Systeme

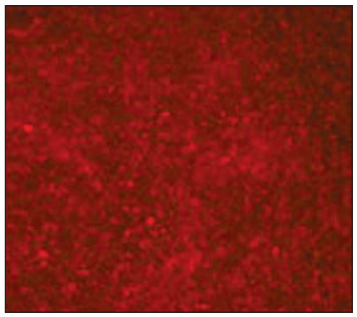
CTC for Visualization of Respiring Bacteria

Polysciences exclusive

Cyanoditolyl Tetrazolium Chloride

CTC or 5-cyano-2,3-ditolyl tetrazolium chloride is useful in the direct visualization and enumeration of respiring plankton or bacteria in environmental samples, food samples and especially water samples. CTC reveals a quantitative methodology for measuring marine bacteria. These methods yield 80% activity in 2 to 10 minutes. CTC is a monotetrazolium redox dye that produces a fluorescent formazan when chemically reduced. CTC is stable for days and fluoresces bright red when excited with long wave UV light greater than 350nm and is readily distinguished from most background fluorescence and autofluorescing abiotic particles which typically emit in the blue to blue-green regions. Cell samples mixed with CTC can be stored refrigerated or frozen in liquid nitrogen for at least 4 weeks without a significant loss of cells.

Cat. #	Description	Size
19292	Cyanoditolyl Tetrazolium Chloride (CTC)	100mg, 1g



E. coli bacteria in CTC

Grover's Corner

Grover's Corner is a new feature in the BioSciences edition of the PolyFacts. In this issue we want to introduce you to our new BioSciences Product Line Manager, Valantou Grover, HT(ASCAP), HTL, PA, MBA. Valantou has an extensive background in every aspect of microscopy, histology, molecular biology, laboratory management and hands on experience in clinical and research settings. She is the person to contact to discuss any of your questions for our BioSciences product line.

Polysciences' Harris Hematoxylin Receives A+ Rating

Polysciences' Mercury-free Harris Hematoxylin was recently tested by Jim Burchette, HT, ASCP, QIHC in an outside independent study to test the quality of our Harris Hematoxylin *(Cat. #24245)* using manual staining techniques. Polysciences' Mercury-free Harris Hematoxylin received an A+ preferred staining rating and outperformed a major leading competitor which only received an A- and demonstrated weak staining. Jim's results show Polysciences' Harris Hematoxylin produces superior results when used in the regressive staining line with acid alcohol and progressive method staining showed pleasing results.

Two types of eosin were used during the study; Polysciences' Eosin Y, 1% alcoholic solution *(Cat. #17269)* and freshly made Eosin-Phloxine working solution following the Armed Forces Institute of Pathology (AFIP) formula. Following the application of Polysciences' Harris Hematoxylin, controls were conventionally counterstained with either Polysciences' Eosin Y, 1% alcoholic solution *(Cat. #17269)* or Eosin-Phloxine working solution. The results demonstrated that the Polysciences' Eosin Y, 1% alcoholic solution allows the end user more control over how the hematoxylin and eosin prefers to be seen by the Pathologist or the Physician's Assistant.

A regressive H&E staining protocol may be preferred by some users in order to achieve crisp nuclear staining results. Progressive H&E staining can demonstrate minor hematoxylin background while regressive H&E staining does not. The progressive method resulted in less eosin contrast and differentiation. Slides were then placed in Polysciences' premixed Lithium Blue *(Cat. #24820)* and some in ammonia water until the sections turned bright blue. The Polysciences' premixed Lithium Blue performed identical to bluing with ammonia water.

The study was performed with set times to reduce the number of variables. Five minutes was used for hematoxylin and 30 seconds for bluing. For more information about Polysciences' full line of hematoxylins and eosin please visit our web site:
www.polysciences.com

Figure 1

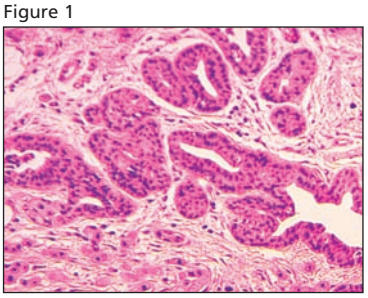


Figure 2

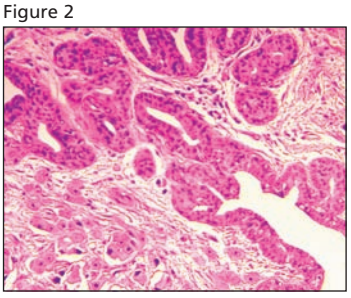


Figure 1: Human Breast Duct Carcinoma, Polysciences' Harris Hematoxylin and Polysciences Eosin Y, 1% alcoholic solution, ammonia water bluing solution. 10X magnification.

Figure 2: Human Breast Duct Carcinoma, Polysciences' Harris Hematoxylin and Eosin-Phloxine working solution. Polysciences Lithium Blue was used as a bluing solution. 10X magnification. *Photos courtesy of Jim Burchette, HT (ASCP), QIHC*

Technical Data Sheet Online
www.polysciences.com/shop/assets/datasheets/70013.pdf

Technical Data Sheet Online
www.polysciences.com/shop/assets/datasheets/192.pdf

Cat. #	Description	Size(s)
24245	Harris Hematoxylin, Acidified (Hg free)	500ml, 1000ml
17269	Eosin Y, 1% alcoholic solution	500ml, 1000ml
24820	Lithium Blue	1 Gallon
24821	Mayers Hematoxylin	500ml, 1 Liter
24242	Gill's Hematoxylin #1 for Cytology	500ml, 1000ml
24243	Gill's Hematoxylin #2, (Histo & Cyto)	500ml, 1000ml
24244	Gill's Hematoxylin #3, (Histology)	500ml, 1000ml
24605	Scott's Bluing Reagent	100ml, 1 Gallon
24819	Ammonium Blue	1 Gallon

NeuroVue® Dye Filters for Neuronal Tract Tracing

What are they?

NeuroVue® Dye Filters are useful tools in several different areas of research including neuronal tract tracing studies up to 3-4 weeks and spectrally compatible with most fluorescent light-absorbing tags.

What do NeuroVue Dye Filters Offer?

- Convenient, ready-to-use coated filter format
- More precise control of dye insertion point
- No messy oils, pastes or hard-to-position crystals
- Diffusion properties comparable to or better than other commercially available neurotracing dyes
- More focal results (e.g. labeling of small sets of axons within pathway)
- Available in multiple colors, including far red, for multi-tract tracing and improved results even in tissues with high myelin expression

How do they work?

NeuroVue® Dye Filters have been found to be useful for tracing neuronal connections in animal tissues fixed in formaldehyde. Like other lipophilic tracers, it readily transfers into plasma membranes in fixed and/or live tissues and diffuses laterally within the membrane, eventually labeling the entire cell body as well as the finest axonal and dendritic branches and allowing visualization of neuronal processes up to several millimeters distant from the point of dye insertion.

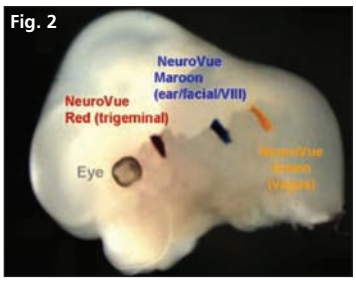
Figure 1: Preparation of NeuroVue® Red micro-strips for use in tissue labeling. Using a dissecting microscope, microscissors are used to cut small triangles from a 1x1cm coated filter square. Magnification ~25X *(Cat. #24835)*

NeuroVue® Dye Filters are provided in coated filter format because insertion of small dye coated filter segments have been shown to be a simple, reliable method for labeling well defined tissue regions, avoiding known artifacts associated with labeling via high pressure microinjection or insertion of dye crystals on a dissecting needle.

Most NeuroVue® Dye Filters fluoresce in the far red and exhibit minimal bleed through into filter windows typically used for visible fluorescing lipophilic tracers such as DiA, DiI, NeuroVue® Red, NeuroVue® Orange or NeuroVue® Jade, making it an excellent choice for multicolor neurotracing studies in sections and/or whole-mount preparations.

Overview of Labeling Strategy *(see Figures 2 & 3)*

- Fix tissue in 4% buffered formaldehyde.
- Initiate labeling by inserting NeuroVue® micro-strip(s) into nerve tract(s) to be traced. The highly lipophilic NeuroVue® dyes transfer from the micro-strip into nerve cell membranes and diffuse along the lipid bilayer in both directions from the insertion site (anterograde and retrograde labeling).
- Incubate tissue in 4% phosphate buffered formaldehyde at 37°C.
- Monitor the progress of dye diffusion using light microscopy and/or fluorescence microscopy.
- When dye(s) have reached the region(s) to be studied, remove NeuroVue® micro-strip(s) and prepare whole mounts or tissue sections for fluorescence imaging.



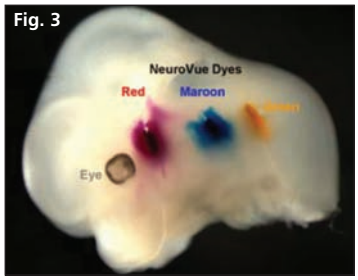


Figure 2: Placement of NeuroVue® micro-strips for multicolor neurotracing. Lateral view of murine head (embryonic day 12.5); with micro-strips placed to obtain central projections of NeuroVue® Red labeled trigeminal nerve, NeuroVue® Maroon labeled facial nerve and NeuroVue® Jade labeled glossopharyngeal nerve. The eye is visible as a brown spot at left (anterior). Magnification ~25X

Figure 3: Monitoring diffusion distance using NeuroVue® dye absorbance. After incubation for 36 h at 37°C, diffusion in all directions from the point of micro-strip insertion is readily visualized using a dissecting microscope (same specimen as Figure 2) Magnification ~25X

Photos courtesy of Drs. Bernd Fritzsch and Lucy Feng (Creighton University)

Advantages of NeuroVue® Technology

- Different fibers can be traced in the same specimen by using fluorescent NeuroVue® dyes that excite and emit in the green, orange, red and/or far red.
- Neuronal connections can be studied in embryos lacking receptors needed for neuronal identification as well as in juveniles and adults. *(Gurung & Fritzsch, J Comp Neurol 479:309-327, 2004; Morris et al., Brain Res 1091:186- 199, 2006; Hsieh & Cramer, J Comp Neurol 497:589-599, 2006)*
- Use of dye-coated filters allows more precise positioning than is possible with crystals or oils, avoids tissue damage caused by high pressure microinjection and provides sharp high resolution images of both afferent and efferent fibers arising at the point of filter insertion. *(Fritzsch et al., Brain Res Bull 66:249-258, 2005)*
- Use of NeuroVue® dyes reduces the complexity of labeling procedures because these dyes have been selected to have similar diffusion rates, allowing simultaneous or near-simultaneous application of different colors in most cases. *(Fritzsch et al., Brain Res Bul, 66: 249-258, 2005)*

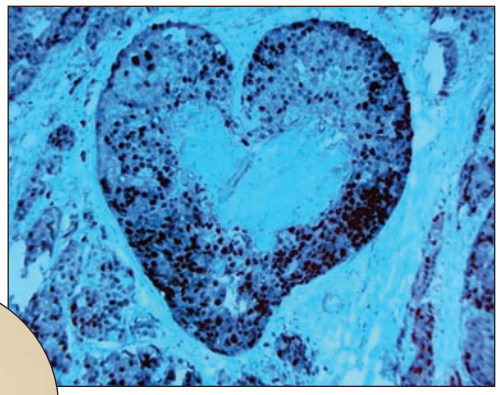
Technical Data Sheet Online
www.polysciences.com/shop/assets/datasheets/770.pdf

Cat. #	Description	Ex. Max	Em. Max	Size
24834	NeuroVue® Maroon	647nm	667nm	1filter
24835	NeuroVue® Red	567nm	588nm	1filter
24836	NeuroVue® Orange	550nm	570nm	1filter
24837	NeuroVue® Jade	494nm	507nm	1filter
24838	NeuroVue® Burgundy	683nm	707nm	1filter
24839	Vannas Scissors, Straight, 5mm blades			1each
24856	Vannas Capsulotomy Scissors, 7mm			1each
24855	Gills A/Welsh Vannas Scissors, Straight, 11mm blades			1each

DAB Chromogen Format Comparison

Tablets, lyophilized and pre-dilute solutions

3-3'-Diaminobenzidine tetrahydrochloride (DAB) is a widely used chromogen for immunoperoxidase and immunoblotting techniques. It is a well accepted technique due to its superior performance over aminoethyl carbozole (AEC). DAB is much more sensitive and it gives a much cleaner background as opposed to AEC. Specimens stained with DAB can be dehydrated in ethanol and cleared in subsequent changes in xylene. However, due to the carcinogenic nature of DAB, laboratories avoid handling DAB in powder form, which brings us to the alternatives: tablets, lyophilized form and solutions or pre-dilutes.



Human breast duct progesterone receptor immunostained with DAB chromogen and counterstained with Light Green.
Photo: Gary A. Stopyra, M.D., Staff Pathologist, Health Network Labs, Lehigh Valley, Allentown, PA

Which version of DAB is safer to handle?

Ready to use solutions or pre-dilutes of DAB are easy to use but can oxidize quickly after manufacturing causing a shift in color to a noticeable, brown or purple color reaction. The benefits of using DAB in lyophilized and reconstituted form include low exposure to the researcher, air and metal. DAB in a tablet form increases the risk of exposure to air, metal and possible exposure to the researcher during the mixing process.

Lyophilized DAB is supplied as one reagent which is convenient to use by puncturing the rubber cap *(see inset photo)* to reconstitute immediately prior to use. After reconstitution, aliquot can be kept at -70°C until needed and may last longer than the oxidized pre-dilute and one that sits at room temperature. Accidental oxidation by the researcher is less likely to occur with the pre-dilute as well as the lyophilized form.

When handling the DAB tablet the researcher must use caution as to limit the use of anything metal, including metal forceps. The metal acts as a catalyst for the oxidation process. Gloves, lab coats and goggles are to be worn at all times in procedures that involve DAB manipulation. All DAB dilutions and aliquots should be done under the fume hood. Reconstituted DAB in the liquid form, may be used immediately or stored as aliquots in 0-20°C. Store lyophilized DAB in the freezer before use.

DAB tablets have ~70% viability with the addition of urea fizzing fillers that may interact with the background staining of the DAB chromogen. An advantage of lyophilized DAB is the strong 99.1% viability and the absence of fillers that interact with background staining. Lyophilized DAB chromogen concentration in the purest form exposes reliable results and less background staining is likely to occur as compared to the tablets and pre-dilutes where handling, temperature and oxidation are factors affecting background staining.

Technical Data Sheet Online
www.polysciences.com/shop/assets/datasheets/164.pdf

Cat. #	Description	Size(s)
04001	DAB-4HCl, Immunochemical Grade	2x25mg in 60ml vials 5x25mg in 60ml vials
04008	DAB-4HCl	5x10mg in 5x30ml serum vials

Questions and answers pertaining to DAB chromogen

Q. How do I oxidize DAB?

A. Some citations have noted that bleach + oxidized DAB mixture might be mutagenic to bacteria. After oxidation of DAB by a solution containing potassium permanganate and sulfuric acid, the resulting solution did not threaten bacteria with mutation. Bleach and potassium permanganate are both active disinfectants, killing bacteria when diluted. One does wonder if testing such substances for mutagenicity is meaningful. Acidified permanganate is recommended to oxidize DAB, this procedure is documented with nearly a full page in the following book: *Lunn G & Sansone EB (1990) Destruction of Hazardous Chemicals in the Laboratory. New York: Wiley, 271 pages.*

Fresh DAB solutions are fairly clear, but moderately brown solutions do not mean they are completely oxidized. Another method to oxidize DAB is to add .12 grams of Horseradish Peroxidase (HRP) and 8ml of 30% hydrogen peroxide to 20 liters of DAB waste, and let it sit overnight. Use a funnel and filter paper to separate any solids and dispose in accordance with local, state and federal laws. Remember to always wear Personal Protective Equipment (PPE) whenever working in the laboratory.

Q. How do I perform a DAB solution viability check?

A. Add a drop of dilute hydrogen peroxide and a source of peroxidase such as a drop of any HRP-labeled reagent. The solution should turn brown in a few seconds. Examine DAB powder (or tablets) for any noticeable solids or dark brown coloration, this may reveal that the DAB has already oxidized and should not be used.

A brown solution of DAB is likely to give brown background staining that has nothing to do with peroxidase activity or immunohistochemistry. The lyophilized version can be kept at -20° C for a few years. Solutions of DAB should be made fresh and used as soon as it has been made, do not allow it to oxidize and turn brown.

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