Diagnostic BioSystems

IHC Made Affordable

DAPI

Catalog Number:

K108

**This data sheet is applicable to all sizes (volume) of product. Actual volume is indicated on vial or bottle

Document #: DS-2069-B
Effective Date: 12/18/2023

Intended Use

For In Vitro Diagnostic Use.

This product is intended for use as a fluorescent counterstain for paraffin-embedded tissue sections, frozen tissue sections, and cell preparations. The DAPI counterstain produces a blue, fluorescent stain, and can be used as a counterstain in conjunction with Fluorescent In Situ Hybridizatin (FISH), immunohistochemistry, and other fluorescent staining procedures.

DAPI's blue emission is convenient for microscopists who wish to use multiple fluorescent stains in a single sample.

Summary and Explanation

DAPI or 4',6-diamidino-2-phenylindole, is a fluorescent stain that binds strongly to adenine–thymine-rich regions in DNA. It is used extensively in fluorescence microscopy. As DAPI can pass through an intact cell membrane, it can be used to stain both live and fixed cells.

Features

- This DAPI solution is optimized for use on formalin-fixed, paraffin-embedded tissue sections.
- The DAPI solution contains an anti-freeze agent so the reagent can be stored at -20 °C without freezing.
- For convenience the DAPI solution can be removed from the freezer and used immediately without the necessity of thawing.
- There is no need to aliquot and freeze small portions.

Known Applications

- Fluorescence *In Situ* Hybridization
- Immunofluorescence with fluorescently labeled antibodies or probes.
- Fluorescent staining methods.

Product Description

This product is provided ready-to-use, and no further dilution is required. This product should be stored at -20 °C. The product contains an anti-freeze agent, so it can be used immediately after removal from storage, and should be returned to storage immediately after use.

When stored at -20°C, the product remains stable up to the expiration date indicated on the label..

Format

Ready-To-Use. Do not dilute.

Volume/UOM

1mL/10mL

Principles of the Procedure

When bound to double-stranded DNA, DAPI has an absorption maximum at a wavelength of 358 nm (ultraviolet) and its emission maximum is at 461 nm (blue). Therefore, for fluorescence microscopy, DAPI is excited with ultraviolet light and is detected through a blue/cyan filter.

Materials Required But Not Provided

Some of the reagents and materials required for IHC are not provided. Pretreatment reagents, detection systems, control reagents and other ancillary reagents are available from Diagnostic BioSystems. Please refer to the Diagnostic BioSystems website at www.dbiosys.com.

Storage and Handling

Store at -20 °C. Do not use after expiration date printed on label. If reagents are stored under conditions other than those specified in the package insert, they must be verified by the user.

Precautions

This product is a single-use, non-sterile, in vitro diagnostic device.

- Wear disposable gloves when handling reagents.
- Specimens, before and after fixation, and all materials exposed to them should be handled as if capable of transmitting infection and disposed of with proper precautions. Never pipette reagents by mouth and avoid contacting the skin and mucous membranes with reagents and specimens. If reagents or specimens come in contact with sensitive areas, wash with copious amounts of water.
- Microbial contamination of reagents may result in an increase in nonspecific staining.
- Incubation times or temperatures other than those specified may give erroneous results. The user must validate any such change.
- Do not use reagent after the expiration date printed on the label.
- The MSDS is available upon request.

Consult OSHA, federal, state or local regulations for disposal of any toxic substances.

Protocol Recommendations:

Manual protocol:

1. After application of the fluorescent probe or dye, wash the cells or tissues with a suitable wash buffer.
2. Add sufficient DAPI stain solution to cover the cells or tissue.
3. Incubate for five minutes, protected from light.
4. Rinse off stain solution with a suitable wash buffer.
5. Mount with an aqueous mounting medium optimized for fluorescence microscopy.

Quality Control

Refer to CLSI Quality Standards for Design and Implementation of Immunohistochemistry Assays; Approved Guideline-Second edition (I/LA28-A2). CLSI Wayne, PA, USA (www.clsi.org). 2011

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Troubleshooting

Problem: No Staining or Low Signal

Primary antibody is not validated for application

Check the supplier information to find out if your primary antibody is recommended for your application. If possible, validate the antibody using a positive control cell line or tissue that expresses the target.

Confirm that the species reactivity of the antibody is compatible with your sample.

Fluorescence is photobleaching during microscopy

Using mounting medium with antifade.

Problem: High Background or Non-Specific Staining
Cell or tissue autofluorescence

Autofluorescence is a major and nearly universal source of background in tissue sections and is present in some primary cells and pigmented cell types.

Include an unstained control to determine the level of autofluorescence in your sample.

Cellular autofluorescence is high in blue wavelengths, so avoid using blue fluorescent conjugates for low expressing targets.

Cross-reactivity of secondary antibody with other antibodies or proteins in sample

Perform staining controls with secondary antibody alone to determine whether the secondary antibody is binding the sample directly.

Antibody concentration too high

If both signal and background are high, antibody concentration may be too high.

Perform a titration of antibody concentration to find the optimal concentration

Warranty

There are no warranties, expressed or implied, which extend beyond this description. Diagnostic BioSystems is not liable for property damage, personal injury, or economic loss caused by this product.

Expected Results

Nuclei of cells and tissues will exhibit a bright blue nuclear stain against a dark background. Other stained elements will exhibit various colors, such as red and green, depending on the specific fluorescent dyes employed.

Limitations of the Procedure

Immunohistochemistry is a complex technique involving both histological and immunological detection methods. Tissue processing and handling prior to immunostaining can also cause inconsistent results. Variations in fixation and embedding or the inherent nature of the tissue may cause variations in results (Nadji and Morales, 1983). Endogenous peroxidase activity or pseudoperoxidase activity in erythrocytes and endogenous biotin may cause non-specific staining depending on detection system used. Tissues containing Hepatitis B surface Antigen (HBsAg) may give a false positive with horseradish peroxidase systems (Omata et al, 1980). Improper counterstaining and mounting may compromise the interpretation of results.

Performance Characteristics

The protocols for a specific application can vary. These include, but are not limited to: fixation, heat-retrieval method, incubation times, and tissue section thickness and detection kit used. Due to the superior sensitivity of these unique reagents, the recommended incubation times and titers listed are not applicable to other detection systems, as results may vary. The data sheet recommendations and protocols are based on exclusive use of Diagnostic BioSystems products. Ultimately, it is the responsibility of the investigator to determine optimal conditions. These products are tools that can be used for interpretation of morphological findings in conjunction with other diagnostic tests and pertinent clinical data by a qualified pathologist.

References

I. Kapuscinski, J. (September 1995). "DAPI: a DNA-specific fluorescent probe". Biotech. Histochem. **70** (5): 220–233.
II. Scott Prahl, DAPI. accessed 2009-12-08.

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