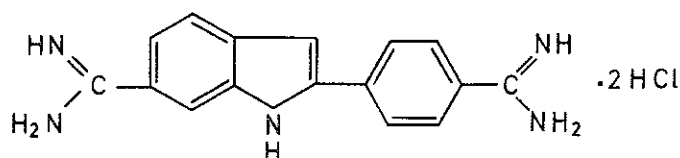




# 18860 D A P I - the new fluorescent DNA stain 10 mg...



4',6-Diamidino-2-phenylindole 2 HCl, research grade

$C_{16}H_{15}N_5$  2 HCl - MW 350.3

$C_{16}H_{15}N_5$  - MW 277.4 79,2%

Maximum of absorption 340 nm, maximum of emission 488 nm.

The biscationic fluorescent dye DAPI was first synthesized by O. Dann et al. (1) as a trypanocide. It has been shown to possess specific binding properties with adenine-thymine-rich DNA of various origins. Thus, DAPI will bind differentially to yeast mitochondrial and nuclear DNA forming highly fluorescent complexes and enhancing the separation of such DNAs in caesium chloride gradients (2).

On adding DAPI to tissue culture cells it was found that it was rapidly taken up into cellular DNA, yielding highly fluorescent nuclei and no detectable cytoplasmic fluorescence. If, however, the cells were contaminated with mycoplasmas, discrete fluorescent foci were readily detected in the cytoplasm and on the surfaces of the cells. Similarly, DAPI staining of cells infected with vaccinia virus revealed characteristic fluorescent virus 'factories' in the cytoplasm (3).

In a similar way large DNA-virus can be stained, phages, bacteria, yeast cells, whereby petite mutants without mitochondria can be clearly distinguished. Furthermore, trypanosomes and mesenchymal mammalian tissues can be stained.

DAPI is soluble in water at approx. 2.5%. Such solutions can be stored in a refrigerator for many weeks. Two- or multivalent anions, such as phosphate or sulfate can precipitate with DAPI at higher concentrations, but not at ordinary working concentrations.

DAPI is very stable in UV-light. For staining on a microscope slide a solution of 0.1 micrograms or  $\mu\text{g}$  DAPI per ml should be left in contact with the specimen for 15-30 minutes at 37°C.

- (1) Dann, O., Bergen, G., Demant, T. & Volz, G., (1971), Ann. Chem. 794, 68
- (2) Williamson, D.H. & Fenell, D.J., 1974, in Methods in Cell Biology (edit. by D. Prescott). Academic Press, New York
- (3) Russel, W.C., Newman, C. & Williamson, D.H., 1975, Natur e, 253, 461-462

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Prices subject to change without notice

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TO ORDER CALL TOLL FREE: **1-800-645-6264** (NEW YORK OFFICE) OR **1-800-255-9378** (WEST COAST OFFICE)

# DAPI Staining of Nuclei

18860

ACCURATE CHEMICAL & SCIENTIFIC CORP.  
300 SHAMES DRIVE  
WESTBURY, NY 11590 USA  
(516) 333-2221  
FAX # (516) 997-4948

DAPI is used to stain DNA and can be used to determine cell cycle information.

1. In most cases the cells will be spun down and the supernatant removed before adding DAPI.
2. Resuspend the pellet in at least 200  $\mu$ l of DAPI. In general you would want the cells at a concentration of  $2 \times 10^6$ /ml of DAPI. The cells can be run on the flow cytometer.
3. The cells can also be frozen for analysis at a later date.

## **Fresh cells in suspension:**

### DNA Content and Cell Cycle Analysis

In brief, cells in suspension are spun and resuspended in a solution of 10 mg/ml 4,6-diamidino-2-phenylindole (DAPI) and 0.1% nonidet P-40 detergent in a Tris buffered saline. The suspension is titrated with a 26 gauge needle and analyzed using a cytometer, with ultraviolet excitation and DAPI emission collected at  $>450$ nm. DNA content and cell cycle are then analyzed.

## **Tissues:**

### DNA Content and Cell Cycle Analysis

In brief, tissue is minced with scalpels in a solution of 10  $\mu$ g/ml 4,6-dimidino-2-phenylindole (DAPI) and 0.1% noidet P-40 detergent in a Tris buffered saline. The supernatant is triturated with a 26 guage needle, filtered through 40  $\mu$ m steel mesh, and analyzed using a cytometer, with ultraviolet excitation and DAPI emission collected at  $>450$ nm. DNA content and cell cylce are then analyzed.

## **Cells from Paraffin:**

### DNA Content and Cell Cycle Analysis

In brief, sections are dewaxed in xylene, gradually rehydrated in a step series of ethanol solutions, and digested in water with 1% pepsin H1.5 37 C for 40 min. The supernatanti is triturated with a 26 guage needle and resuspended in an isotonic ph 7.4 buffered solution with 0.1% nonidet P-40 dtergent, 10  $\mu$ g/ml diamidino-2-phenlindole (DAPI) and 1% RNase, and filtered through 40  $\mu$ m steel mesh. The analysis is performed on a cytometer using UV excitation. 50,000 cells are analyzed, if available, and in all cases acceptable histograms contained at least 10,000 cells and a coefficient of variation below 6.0%. DNA content and cell cylce are then analyzed.

## **DAPI Working Solution**

To 500 ml dd H<sub>2</sub>O add:

1. 8.5g NaCl (final conc. = 146 mM)
2. 1.2g Tris Base (final conc. = 10 mM)

Adjust pH to 7.4 with HCl

Add:

1. 4 ml of 500 mM CaCl<sub>2</sub> soln. (final conc. = 2 mM)
2. 44 ml of 500 mM MgCl<sub>2</sub> soln. (final conc. = 22 mM)
3. 50 mg (0.05g) BSA
4. 1 ml nonident P-40 (NOT the same as NP-40) detergent. (final conc. = 0.1%)
5. 10 mg DAPI (4,6-diamidino-2-phenylindole) powder. (final conc. = 10  $\mu$ g/ml)  
(Accurate Chemical Company, catalog # 18860; NO SUBSTITUTES!)
6. 100 ml DMSO (final conc. = 10%)

Add dd H<sub>2</sub>O to final volume of 1L.

Store in dark or foil wrapped bottle at 2-6 C.

# ACCU-SPECS SHEET



## M A T E R I A L   S A F E T Y   D A T A   S H E E T

SECTION 1. - - - - - CHEMICAL IDENTIFICATION- - - - -  
CATALOG #: 18860  
NAME: 4',6-DIAMIDINO-2-PHENYLINDOLE  
DIHYDROCHLORIDE HYDRATE

SECTION 2. - - - - - COMPOSITION/INFORMATION ON INGREDIENTS - - - - -  
CAS #: 28718-90-3  
MF: C16H15N5  
EC NO: 249-186-7  
HAZARDOUS INGREDIENTS  
CONTAINS ACETONE, CHEMICAL ABSTRACTS REGISTRY NUMBER 67-64-1.

SECTION 3. - - - - - HAZARDS IDENTIFICATION - - - - -  
LABEL PRECAUTIONARY STATEMENTS  
IRRITANT  
IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN.  
TARGET ORGAN(S):  
LIVER  
KIDNEYS  
IN CASE OF CONTACT WITH EYES, RINSE IMMEDIATELY WITH PLENTY OF  
WATER AND SEEK MEDICAL ADVICE.  
WEAR SUITABLE PROTECTIVE CLOTHING.

SECTION 4. - - - - - FIRST-AID MEASURES- - - - -  
IN CASE OF CONTACT, IMMEDIATELY FLUSH EYES OR SKIN WITH COPIOUS  
AMOUNTS OF WATER FOR AT LEAST 15 MINUTES WHILE REMOVING CONTAMINATED  
CLOTHING AND SHOES.  
IF INHALED, REMOVE TO FRESH AIR. IF NOT BREATHING GIVE ARTIFICIAL  
RESPIRATION. IF BREATHING IS DIFFICULT, GIVE OXYGEN.  
IF SWALLOWED, WASH OUT MOUTH WITH WATER PROVIDED PERSON IS CONSCIOUS.  
CALL A PHYSICIAN.  
REMOVE AND WASH CONTAMINATED CLOTHING PROMPTLY.

SECTION 5. - - - - - FIRE FIGHTING MEASURES - - - - -  
EXTINGUISHING MEDIA  
CARBON DIOXIDE, DRY CHEMICAL POWDER OR APPROPRIATE FOAM.  
WATER SPRAY.  
SPECIAL FIREFIGHTING PROCEDURES  
WEAR SELF-CONTAINED BREATHING APPARATUS AND PROTECTIVE CLOTHING TO  
PREVENT CONTACT WITH SKIN AND EYES.  
UNUSUAL FIRE AND EXPLOSIONS HAZARDS  
EMITS TOXIC FUMES UNDER FIRE CONDITIONS.

SECTION 6. - - - - - ACCIDENTAL RELEASE MEASURES- - - - -  
WEAR RESPIRATOR, CHEMICAL SAFETY GOGGLES, RUBBER BOOTS AND HEAVY  
RUBBER GLOVES.  
SWEEP UP, PLACE IN A BAG AND HOLD FOR WASTE DISPOSAL.  
AVOID RAISING DUST.  
VENTILATE AREA AND WASH SPILL SITE AFTER MATERIAL PICKUP IS COMPLETE.

SECTION 7. - - - - - HANDLING AND STORAGE- - - - -  
REFER TO SECTION 8.

SECTION 8. - - - - - EXPOSURE CONTROLS/PERSONAL PROTECTION- - - - -  
CHEMICAL SAFETY GOGGLES.  
MECHANICAL EXHAUST REQUIRED.  
SAFETY SHOWER AND EYE BATH.  
COMPATIBLE CHEMICAL-RESISTANT GLOVES.  
NIOSH/MSHA-APPROVED RESPIRATOR.  
DO NOT BREATHE VAPOR.  
DO NOT GET IN EYES, ON SKIN, ON CLOTHING.  
AVOID PROLONGED OR REPEATED EXPOSURE.  
WASH THOROUGHLY AFTER HANDLING.  
IRRITANT.  
KEEP TIGHTLY CLOSED.  
STORE IN A COOL DRY PLACE.

SECTION 9. - - - - - PHYSICAL AND CHEMICAL PROPERTIES - - - - -  
APPEARANCE AND ODOR  
YELLOW POWDER

SECTION 10. - - - - - STABILITY AND REACTIVITY - - - - -  
STABILITY  
STABLE.  
INCOMPATIBILITIES  
STRONG OXIDIZING AGENTS  
HAZARDOUS COMBUSTION OR DECOMPOSITION PRODUCTS  
TOXIC FUMES OF:  
CARBON MONOXIDE, CARBON DIOXIDE  
NITROGEN OXIDES .

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HAZARDOUS POLYMERIZATION  
WILL NOT OCCUR.

SECTION 11. - - - - - TOXICOLOGICAL INFORMATION - - - - -

ACUTE EFFECTS

MAY BE HARMFUL BY INHALATION, INGESTION, OR SKIN ABSORPTION.  
CAUSES EYE AND SKIN IRRITATION.

MATERIAL IS IRRITATING TO MUCOUS MEMBRANES AND UPPER  
RESPIRATORY TRACT.

CAUSES DERMATITIS.

TARGET ORGAN(S):

LIVER, KIDNEYS

TO THE BEST OF OUR KNOWLEDGE, THE CHEMICAL, PHYSICAL, AND  
TOXICOLOGICAL PROPERTIES HAVE NOT BEEN THOROUGHLY INVESTIGATED.

SECTION 12. - - - - - ECOLOGICAL INFORMATION - - - - -

DATA NOT YET AVAILABLE.

SECTION 13. - - - - - DISPOSAL CONSIDERATIONS - - - - -

DISSOLVE OR MIX THE MATERIAL WITH A COMBUSTIBLE SOLVENT AND BURN IN A  
CHEMICAL INCINERATOR EQUIPPED WITH AN AFTERBURNER AND SCRUBBER.

OBSERVE ALL FEDERAL, STATE AND LOCAL ENVIRONMENTAL REGULATIONS.

SECTION 14. - - - - - TRANSPORT INFORMATION - - - - -

CONTACT SIGMA CHEMICAL COMPANY FOR TRANSPORTATION INFORMATION.

SECTION 15. - - - - - REGULATORY INFORMATION - - - - -

EUROPEAN INFORMATION

IRRITANT

R 36/37/38

IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN.

S 26

IN CASE OF CONTACT WITH EYES, RINSE IMMEDIATELY WITH PLENTY OF  
WATER AND SEEK MEDICAL ADVICE.

S 36

WEAR SUITABLE PROTECTIVE CLOTHING.

TLV AND SOURCE

FOR ACETONE:

ACGIH TLV-TWA: 750 PPM (1780 MG/M3); STEL 1000 PPM (2380 MG/M3).

OSHA PEL: 8H TWA 750 PPM (1800 MG/M3); STEL 1000 PPM (2400 MG/M3).

SECTION 16. - - - - - OTHER INFORMATION - - - - -

THE ABOVE INFORMATION IS BELIEVED TO BE CORRECT BUT DOES NOT PURPORT TO  
BE ALL INCLUSIVE AND SHALL BE USED ONLY AS A GUIDE.

SHALL NOT BE HELD LIABLE FOR ANY DAMAGE RESULTING FROM HANDLING  
OR FROM CONTACT WITH THE ABOVE PRODUCT.

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