



NuCycl™ DAPI Kit Summary Factsheet

NUCYCL DAPI KIT CONTENTS:

CAT.# 7034

- | | | |
|----|--|------------|
| 1. | 1 vial containing 50 ml of NuCycl DAPI | 50 Tests |
| 2. | 50 Improved NuCycl Sample Preparation Filters | 50 Filters |
| 3. | 1 Instruction Manual | 1 manual |

###

MATERIALS REQUIRED BUT NOT PROVIDED BY EXALPHA:

Materials needed for Exalpa's NuCycl DAPI

- Flow Cytometry Instrument or DNA Analyzer
- Test Tubes compatible with Instrument or Analyzer
- Calibrated Liquid handling device or pipette capable of accurately delivering 1 ml of NuCycl reagent to samples and standards.

FLUORESCENCE INFORMATION AND EQUIPMENT SPECIFICATIONS:

- | | |
|-------------|--------------------------|
| NuCycl DAPI | - Excites at 365 nm. |
| | - Emits at 420 - 470 nm. |

###

ASSAY PROCEDURE:

<<<< **NuCycl DAPI** >>>>

NuCycl DAPI (4', 6 - diamidino -2 phenylindole - 2 HCL) - DNA specific stain procedure. NuCycl DAPI allows the rapid isolation of nuclei from cells and tissues. NuCycl DAPI stains only DNA and DOES NOT stain RNA.

Assay Procedure: The following procedure is recommended for the preparation of cells or tissues for DNA Flow Cytometry. Set instrument or analyzer for DNA/RNA analysis. See instrument manufacturers instructions for proper DNA/RNA procedure.

Note: Exalpa recommends that the NuCycl Sample Preparation filters be used for the preparation of all samples and standards to be run. NuCycl Sample Preparation Filters remove background noise and allow for tighter CV's and hence better sensitivity than would otherwise be possible.

Fresh or Frozen Tissue Procedure

1. Fresh or frozen tissues are trimmed to remove necrotic or normal tissue components, e.g. fat, connective components, etc.
 2. Tissues are teased with two small blades (No. 10) scalpels in a small petri dish (35 - 50 mm diameter) with NuCycl DAPI at a relative concentration of 2-3 mm³ tissue/one ml of NuCycl DAPI. Tissues samples as small as 2 mm in diameter may be used with 1 ml of NuCycl DAPI.
 3. After a minimum of 30 seconds of incubation at room temperature, the samples are pipetted in a test tube and stored on ice.
 - 4- The stained nuclei and debris are separated from each other using the specially designed filter tubes provided. Place filter tube on ice and add the sample to the top portion of the funnel. Remove the filter cap from tube and store on ice.
- NOTE:** It is essential that Exalpa's specially designed filter tubes be used for all applications in order to achieve optimal results.

For research use only. Not for use in human diagnostics or therapeutics.

5. The sample is now ready for immediate DNA flow cytometric analysis.

###

KIT STORAGE AND EXPIRATION DATING: NuCycl DAPI should be stored at 4-8 ° C.

###

REFERENCES:

1. Thornthwaite, J.T., Thomas, R.A. High - Resolution DNA Measurements Using the Nuclear Isolation Medium, DAPI, with the RATCOM Flow Cytometer. *Methods in Cell Biology* 33:111-119, 1990.
2. Hornicek, F.J., Thornthwaite, J.T., Malinin, G.I., Seckinger, D., and Malinin, T.I. Evaluation of 4,6 - diamidino-2-phenylindole (DAPI) for Flow Cytometry of Blastogenesis.
3. Thornthwaite, J.T., et al. A Review of DNA Flow Cytometric Preparatory and Analytical Methods. *Immunocytochemistry in Tumor Diagnosis*. Ed. J. Russo. Martinus Nijhoff: Boston pp. 380-398, 1985.
4. Lee, G.M., Thornthwaite, J.T., and Rasch, E.M. Cytometric Comparisons of DNA Levels in Neuronal and Glial Cells of the Cerebellum. *Cell Biochem. Function*. 33:262-270, 1985.
5. Lee, G.M., Thornthwaite, J.T., and Rasch, E.M. Picogram Per Cell Determination of DNA by Flow Cytofluorometry. *Anal. Biochem.* 137:221-226, 1984.
6. Coulson, P.B., Thornthwaite, J.T., Wooley, T., Seckinger, D., and Sugarbaker, E.V. Prognostic Indicators Including DNA Histogram Type Receptor Content, and Staging Related to Human Breast Cancer Patient Survival. *Cancer Research*. 44:4187-4196, 1984.
7. Youngberg, G.A., Thornthwaite, J.T., and Fanzus, D. Cytologically Malignant Squamous-Cell Carcinoma of the Penis. *J. Dermatol. Surg. Oncol.* 9:474-479, 1983.
8. Evans, D., Thornthwaite, J.T., Ng A., and Sugarbaker, E.V. DNA Flow Cytometry of Human Pleural Effusions: Comparison with pathology for the Diagnosis of Malignancy. *Anal. Quant. Cyto.* 5:19-27, 1983.
9. Thornthwaite, J.T., Sugarbaker, E.V., Temple W.J. Preparations of Tissues for DNA Flow Cytometric Analysis. *Cytometry*. 1:229-237, 1980.
10. Allen, L.M., Thornthwaite, J.T. Studies on the Pharmacology and Cytokinetics of Imidazole Pyrazole (NSC-51143) with p815 Mastocytoma Cells. *Cancer Research* 40:4059-1063, 1980.
11. Thornthwaite, J.T., Allen, L.M. The Effect of Glutamine Analog, AT-125, on the Cell Cycle of MCF-7 and BT-20 Human Breast Carcinoma Cells Using DNA Flow Cytometry. *Res. Comm. Chem. Path.* 29:393-396, 1980.
12. Temple, W.J., Sugarbaker, E.V., Thornthwaite, J.T., Hensley, G.T., Ketcham, A.S. Correlation of Cell Cycle Analysis with Duke's Staging in Colon Cancer Patient. *J. Surg. Res.* 28:314-318, 1980.
13. Sugarbaker, E.V., Thornthwaite, J.T., Temple, W.J., Ketcham, A.S. Flow Cytometry: General Principals and Applications to Selected Studies in Tumor Biology. *Int. Adv. Surg. Oncol.* 2:125-153.

For research use only. Not for use in human diagnostics or therapeutics.

Exalpha Biologicals, Inc., 86 Rosedale Rd. Watertown, MA 02472
Tel: 800.395.1137 or 617.924.3400, Fax: 866.924.5100 or 617.924.5100, Web:www.exalpha.com