Hoechst 33342
Catalog #639

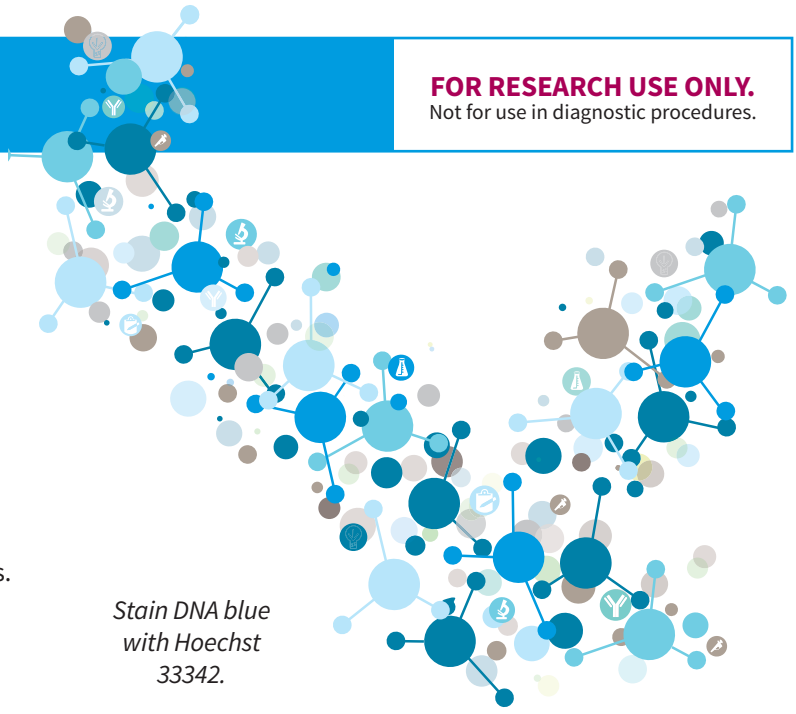
FOR RESEARCH USE ONLY.
Not for use in diagnostic procedures.

INTRODUCTION

Hoechst 33342 is a popular cell-permeant, blue fluorescent nuclear stain. It is used to visualize the nuclei of living or fixed cells and tissues and is often used to distinguish condensed, pyknotic nuclei in apoptotic cells.

Hoechst 33342 emits blue fluorescence when bound to double stranded DNA. It is slightly more membrane permeant than the Hoechst 33258 analog. Hoechst 33342 may be used to identify healthy or apoptotic nuclear morphology and for cell cycle studies.

Each vial of Hoechst 33342 contains 1 mL of aqueous solution at 200 µg/mL (catalog #639). It is ready to use: just add it to the cell culture media at 0.5%, incubate 10-20 minutes, and analyze. Hoechst 33342 can be excited with a xenon or mercury-arc lamp or with a UV laser, and may be used in flow cytometry systems utilizing UV excitation sources. When bound to nucleic acids, the maximum absorption is 350 nm and the maximum emission is 461 nm. It is revealed under a microscope using a UV-filter with excitation set at 365 nm and emission set at 480 nm. Hoechst 33342 is for research use only. Not for use in diagnostic procedures.



Stain DNA blue with Hoechst 33342.

HOW TO USE

Hoechst 33342 is supplied ready-to-use at 200 µg/mL.
To stain cellular nuclei:

1. Add Hoechst 33342 to the cell sample media at 0.5% v/v. For example, add 1.5 µL Hoechst to 300 µL of cells.
2. Incubate 10-20 minutes at room temperature.
3. Visualize with a fluorescence microscope by using a UV excitation and blue emission filter. The blue Hoechst stain fluoresces at 461 nm.
4. Alternatively, cells may be analyzed with a flow cytometer using a UV excitation source.
5. When bound to dsDNA, the maximum absorption is 350 nm and the maximum emission is 461 nm.

SPECIFICATIONS

- 1 mL at 200 µg/mL
- Pale green liquid
- pH 4.0 ± 1.0

STORAGE

- 2-8°C
- Shelf-life up to 24 months when refrigerated and protected from light.

SAFETY

- See Safety Data Sheet (SDS) for any warnings.
- SDS available at www.immunochemistry.com and by calling ICT at 952-888-8788 and 800-829-3194.
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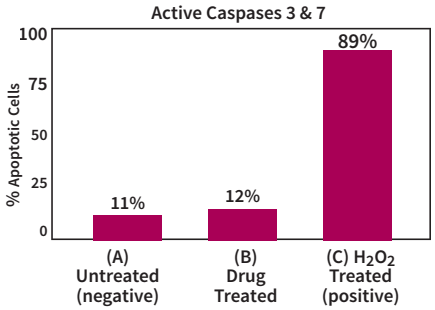
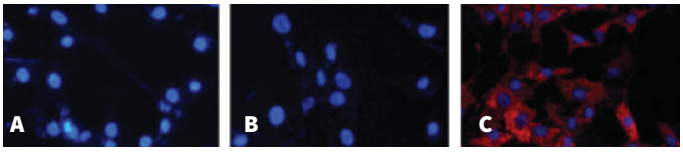
FIGURE 1: QUANTIFYING THE APOPTOTIC EFFECTS OF A DRUG AND CONTROLS ON PULMONARY ARTERY SMOOTH MUSCLE CELLS

ICT's Magic Red® fluorogenic caspase-3/7 substrate, MR-$(DEVD)_2$ (catalog #936), and Hoechst 33342 were used to quantify apoptosis via caspase-3/7 activity in human pulmonary artery smooth muscle cells (PASMC). Cells were treated with: (A) a negative control condition; (B) a drug that inhibits proliferation of PASMCs; or (C) 1 mM H_2O_2 as a positive control to induce apoptosis. Cells were then labeled with MR-$(DEVD)_2$ to detect caspase-3/7 and with Hoechst to stain nuclei blue. Apoptotic cells with active caspases 3 and 7 stained red with cleaved MR-$(DEVD)_2$ and blue with Hoechst. They have less intense blue nuclei (Hoechst) than healthy cells, which have bright blue nuclei. Only 11% of untreated cells (A) and 12% of drug-treated cells (B) were apoptotic (red) compared with 89% of cells treated with H_2O_2 (C). Although this drug inhibits proliferation of PASMCs, it does not induce apoptosis. Research done by Dr. Frederic Perros, et al., Universite Paris-Sud 11; INSERM U764, Clamart; INSERM U841, Hopital Henri-Mondor, Creteil, France.





ImmunoChemistry TECHNOLOGIES LLC
ImmunoChemistry Technologies, LLC
9401 James Ave. S. #155, Bloomington, MN 55431 USA 800-829-3194
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FIGURE 2: MICROSCOPY ANALYSIS OF THP-1 SUSPENSION CELLS

Human monocytic leukemia THP-1 cells were dually stained with Hoechst 33342 (catalog #639), a blue DNA stain, and ICT's green FAM-FLICA® poly-caspase inhibitor reagent, FAM-VAD-FMK (catalog #92). Cells were incubated with 1 µM staurosporine (catalog #6212), for 3 hours at 37°C to induce apoptosis. Cells were labeled with FAM-VAD-FMK for 60 minutes at 37°C. After wash steps, Hoechst stain was added and incubated for 5 minutes. Wet-mount slides were prepared and two photos were taken and superimposed. Nuclear staining by Hoechst (blue) was revealed using a UV-filter (excitation at 365 nm, emission at 480 nm). Caspase activity (green) was detected using a band pass filter (excitation at 488 nm, emission at 520 nm). Only one of the three cells pictured is apoptotic (middle) – it is stained positive (green) for caspase activity with FAM-VAD-FMK. It also displays many bright blue spots from the Hoechst stain, indicating that the cell is beginning to die. The lack of green staining and the concentrated blue DNA in the lower right cell indicate it is alive (not apoptotic). The upper left cell is necrotic (scattered blue, no green). Data courtesy of Dr. Brian W. Lee, ICT.

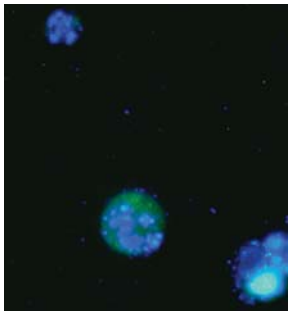


FIGURE 3: DUAL STAINING OF MCF-7 BREAST CANCER CELLS

Human breast cancer MCF-7 cells were dually stained with ICT's Hoechst 33342 nuclear stain (catalog #639) and Magic Red®-$(DEVD)_2$ fluorogenic caspase-3/7 substrate (catalog #936). MCF-7 cells were exposed to 0.15 µM camptothecin (catalog #6210) for 24 hours at 37˚C, then stained with MR-$(DEVD)_2$ for 30 minutes at 37˚C, washed twice in PBS, and supravitally stained with 1 µg/mL Hoechst stain for about 10 minutes. Using the Nikon Microphot FXA system with multi-wavelength filter pairs (UV for Hoechst stain and green light for MR-$(DEVD)_2$), apoptotic cells bearing orange-red lysosomal bodies with less intense blue nuclei are intermixed with non-apoptotic cells bearing bright blue nuclei and absent or reduced orange-red lysosomal staining.
Photo provided by Dr. Zbigniew Darzynkiewicz at Brander Cancer Research Center Institute, New York.

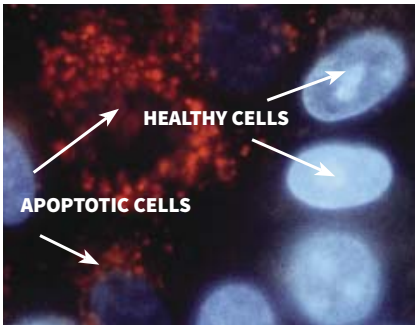
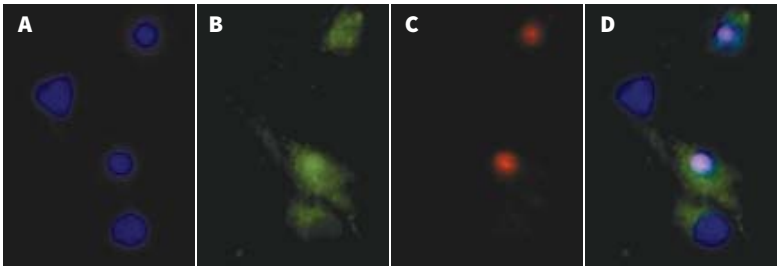


FIGURE 4: CELL DEATH IN PRIMARY RAT HIPPOCAMPAL NEURONS

ICT's Hoechst stain (catalog #639), FAM-FLICA® caspase-3/7 inhibitor reagent (catalog #94), and propidium iodide (PI, catalog #638) were used to assess cell death in primary rat hippocampal neurons. Subjects were first-generation descendants of Sprague–Dawley albino rats. Hippocampi from postnatal day 0 male pups were used for primary cultures of hippocampal neurons. Cells were plated on 25-mm poly-l-lysine-coated coverslips at 300,000 cells per coverslip and were analyzed at 4 or 8 days *in vitro*. (A) Four cells are revealed by labeling the DNA blue with Hoechst. (B) Three apoptotic cells fluoresce green with FLICA® FAM-DEVD-FMK. (C) Two of the apoptotic cells have progressed to secondary necrosis and are in the late stages of apoptosis. They have compromised membranes (PI-positive, C) and also have active caspases (FLICA®-positive, B). In the composite image (D), only one cell out of four is healthy (blue) and the other three cells are apoptotic (green). Data courtesy of Dr. Z. Kahraman Akozer, University of Maryland.



Thank you for using Hoechst 33342!
If you have any questions, or would like to share your data, please contact us at help@immunochemistry.com.

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ImmunoChemistry Technologies, LLC gratefully acknowledges the significant contributions made by one of its founders, Brian W. Lee, Ph.D in the development of this product, including the creation and illustration of its strategy and protocol.

ImmunoChemistry Technologies, LLC
9401 James Ave. S. #155, Bloomington, MN 55431 USA 952-888-8788, 800-829-3194
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