



Description: ViaStain™ Hoechst 33342

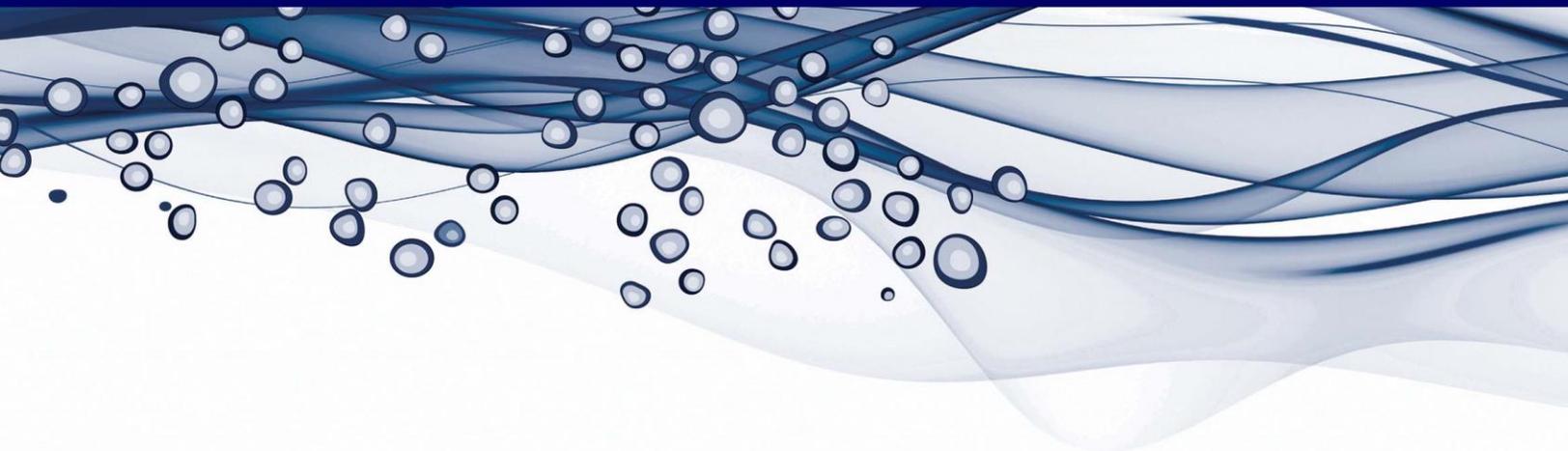
Product Number:

Full Kit: CS1-0128-5mL

Sample Kit: CS1-0128-S (Not available for purchase)

Instrument (s): Celigo, Vision CBA, Spectrum

Product Insert: ViaStain™ Hoechst 33342



This product is for RESEARCH USE ONLY and is not approved for diagnostic or therapeutic use.

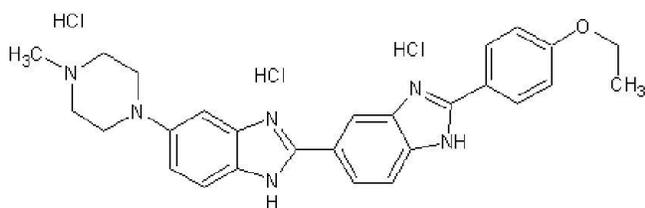
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Description

The Hoechst stains are a family of fluorescent stains for labeling DNA in fluorescence microscopy. Because these fluorescent stains label DNA, they are also commonly used to visualize nuclei and mitochondria. Two of these closely related bis-benzimides are commonly used: Hoechst 33258 and Hoechst 33342. Both dyes are excited by ultraviolet light at around 350 nm, and both emit blue/cyan fluorescence light with an emission maximum at 461 nm. The Hoechst stains may be used on live or fixed cells, and are often used as a substitute for another nucleic acid stain, DAPI.

The key difference between them is that the additional ethyl group of Hoechst 33342 makes it more lipophilic, and thus more readily to cross intact cell membranes. In some applications, Hoechst 33258 is significantly less permeant than Hoechst 33342. The products may be used in fluorescence microscopy, microplate, cuvette, and flow cytometry applications.



Molecular Weight: 561.93

Excitation = 350 nm; Fluorescence = 461 nm

Materials

Materials Supplied

1. CS1-0128-5mL
2. CS1-0128-S (20 µL)

Materials Required

1. Phosphate Buffer Saline (PBS)
2. Pipette
3. 96 well plates
4. Celigo 4 or 5 channel or Vision CBA or Spectrum

Procedure for one 96-well plate on Celigo

Procedure for Staining and Imaging of Adherent and Suspension Cells:

- 1.1. Plate cells in 96-well at 10,000 cells/well in a volume of 100 μ L/well
- 1.2. Incubate overnight in 37°C 5% CO₂
- 1.3. Into 10 mL of 1x PBS buffer add 8 μ L of stock 20 mM Hoechst 33342 to make a 2x staining solution
- 1.4. Pipet 100 μ L of the 2x staining solution from step 1.3 into each well containing 100 μ L of media and cells
- 1.5. Incubate for 30 minutes at in 37°C 5% CO₂
- 1.6. Image on Celigo using the defined imaging conditions below
- 1.7. Image on Celigo with Expression Analysis: Target 1+2 Independent

| Instrument | Channel | Illumination | Exposure | Stain Concentration |
|------------|-----------------|--------------|----------------|---------------------|
| Celigo | Target 1 | Bright field | AEAG (auto) | N/A |
| Celigo | Target 2 (HWAF) | Blue | 80,000 μ s | 8 μ M |

Procedure for Cellometer Vision CBA or Spectrum

Procedure for Staining and Imaging of Adherent and Suspension Cells:

- 1.1. Trypsinize adherent cells, spin down at 1200 rpm for 5 minutes and decant media
 - 1.1.1. If cells are a suspension cell line, spin down and decant the media
- 1.2. Re-suspend cells in 950 μ L of PBS
- 1.3. Stain cells with Hoechst at a final [10 μ M]. First make an intermediary concentration of Hoechst by pipetting 5 μ L of Hoechst into 495 μ L of PBS producing a concentration of 200 μ M – Tube A
- 1.4. From Tube A pipette 50 μ L of diluted Hoechst into 950 μ L of cells in PBS, producing the final [10 μ M]
- 1.5. Incubate cells for 30 minutes in the dark at room temperature
- 1.6. After staining, load 20 μ L of cells into a counting chamber and image using Cellometer Vision CBA
- 1.7. On Cellometer Vision CBA: Make sure Fluorescent Optic Module (FOM) VB-450-302 is installed
- 1.8. On Cellometer Spectrum: Make sure Fluorescent Optic Module (FOM) S1-452-365 is installed
- 1.9. Image the sample based on the parameters shown below

| Instrument | Channel | Illumination | Exposure | Optics Module | Stain Concentration |
|------------|---------|--------------|----------|---------------|---------------------|
| Vision CBA | Ch2 | Blue | 1000 ms | VB-450-302 | 10 μ M |
| Spectrum | Ch2 | Blue | 500 ms | S1-452-365 | 10 μ M |

Storage and Handling

Store ViaStain™ Hoechst 33342 between -16°C to -24°C. Avoid repeated freeze/thaw cycles. Please consult the Safety Data Sheet for more safety information, found on www.nexcelom.com/Products.

Warranty

This product is for RESEARCH USE ONLY and is not approved for diagnostic or therapeutic use. Product is warranted to meet the specifications outlined in the Certificate of Analysis when stored and used according to the manufacturer's instructions. No other warranty, expressed or implied (such as merchantability, fitness for a particular purpose, or non-infringement) is granted. Warranty is valid until the expiration date stated on the product label. If no expiration is listed, the warranty is valid for 12 months from the date of product receipt.

Warranty will be void if product is stored incorrectly, the recommended protocol is not followed, or the product is used for a different application.

Ordering Information

For orders shipping to destinations in the United States:

- When ordering with a Purchase order
 - Fax a copy of your order to 978-327-5341
 - Email a copy of your order to sales@nexcelom.com
- When ordering with a Credit Card
 - Visit www.shop.nexcelom.com and place your order

For orders shipping to destinations outside the United States:

- Contact your local distributor or Nexcelom Representative to place your order