ab228551 Hoechst 33342 Staining Dye Solution

For labeling DNA in fluorescence microscopy. This product is for research use only and is not intended for diagnostic use.

For overview, typical data and additional information please visit: www.abcam.com/ab228551
(use abcam.cn/ab228551 for China, or abcam.co.jp/ab228551 for Japan)

Overview

Hoechst 33342 Staining Dye Solution (ab228551) is a fluorescent stain for labeling DNA in fluorescence microscopy. This product may be used in fluorescence microscopy, microplate, cuvette, and flow cytometry applications. It can also be used to detect the contents of a sample DNA by plotting a standard emission-to content curve.

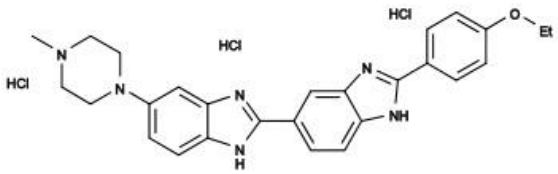

Figure 1. Chemical structure of Hoechst 33342.

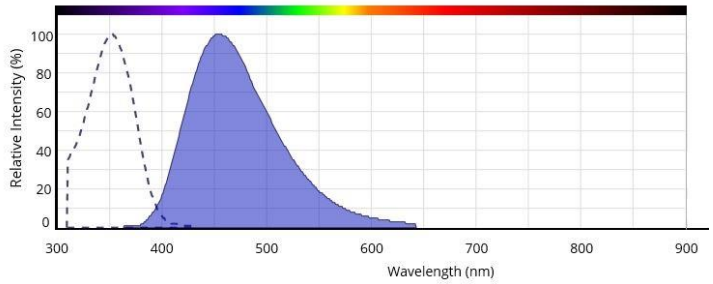

Figure 2. Spectrum of Hoechst 33342.

Materials Supplied and Storage

Store kit at -20°C in the dark immediately on receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.

Item	Quantity	Storage temperature (before prep)
Hoechst 33342 (20 mM Solution in Water)	5 mL	-20°C

Materials Required, Not Supplied

– Buffered salt solutions or media, with optimal dye binding at pH 7.4.

1. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

Hoechst 33342 (20 mM Solution in Water): Ready to use as supplied.

2. Assay Procedure

– Equilibrate all materials and prepared reagents to room temperature just prior to use and gently agitate.
– The following procedure can be adapted for most cell types. Growth medium, cell density, the presence of other cell types and other factors may influence staining.
– Residual detergent on glassware may also affect real or apparent staining of many organisms, causing brightly stained material to appear in solutions with or without cells present.

2.1 Pellet cells by centrifugation.
2.2 Resuspend the cells in buffered salt solutions or media, with optimal dye binding at pH 7.4.
2.3 Adherent cells in culture may be stained *in situ* on cover slips or in the cell culture wells.
2.4 Add Hoechst stain using the concentrations between 0.5 and 5 µM and incubate it for 15 to 60 minutes as a guide.
2.5 In initial experiments, it may be best to try several dye concentrations over the entire suggested range to determine the concentration that yields optimal staining.

3. Calculations

Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiply the concentration found by the appropriate dilution factor. Use only the linear rate for calculation.

3.1 Subtract the mean absorbance/fluorescence value of the blank (Standard #1) from all standard and sample readings. This is corrected absorbance (OD)/fluorescence (RFU).
3.2 Average the duplicate reading for each standard and sample.
3.3 Plot the corrected standard curve readings and draw the line of the best fit to construct the standard curve (most plate reader software or Excel can do this step). Calculate the trend line equation based on your standard curve data (use the equation that provides the most accurate fit).
3.4 Calculate ΔOD/ΔRFU signal in the sample as follows:

$\mathbf{\Delta OD = A_{HC} - A_{Sample}}$

$\mathbf{\Delta RFU = RFU_{HC} - RFU_{Sample}}$

Where "HC" is the reading of the sample High Control, "Sample" is the reading of the sample.
3.5 Apply the ΔOD/ΔRFU to H_2O_2 Standard Curve (colorimetric or fluorometric as per assay) to get B nmol of H_2O_2 decomposed by catalase during the 30 min reaction.
3.6 Catalase activity (nmol/min/mL or mU/mL) in the test samples is calculated as:

$$Catalase\ Activity = \left(\frac{B}{30\ x\ V}\right) * D$$

Where:
B = amount of H_2O_2 in sample well calculated from standard curve (nmol).
30 = Catalase reaction time (minutes) – see Step 4.3.2 or 5.3.3.
V = preoriginal sample volume added into the reaction well (mL).
D = sample dilution factor if sample diluted further to fit within standard curve reading.
1 Unit Catalase activity = amount of catalase that will decompose 1.0 µmol of H_2O_2 per minute at pH 4.5 at 25°C.

Interferences These chemicals or biological materials will cause interferences in this assay causing compromised results or complete failure:
– Reducing agents: DTT or β-mercaptoethanol present at > 5 µM.

General troubleshooting points can be found at www.abcam.com/assaykitguidelines.

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