ab228550
Hoechst 33258
Staining Dye Solution

For labeling DNA in fluorescence microscopy.

This product is for research use only and is not intended for diagnostic use.
# Table of Contents

1. Overview  
2. Materials Supplied and Storage  
3. Materials Required, Not Supplied  
4. General guidelines, precautions, and troubleshooting  
5. Reagent Preparation  
6. Assay Procedure  
7. FAQs / Troubleshooting  
8. Notes
1. Overview

Hoechst 33258 Staining Dye Solution (ab228550) 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.

![Chemical structure of Hoechst 33258.](image)

**Figure 1.** Chemical structure of Hoechst 33258.

![Spectrum of Hoechst 33258.](image)

**Figure 2.** Spectrum of Hoechst 33258.
Pellet cells by centrifugation.

Resuspend the cells in buffered salt solutions or media, with optimal dye binding at pH 7.4.

Add Hoechst stain using concentrations between 0.5 and 5 µM and incubate for 15 to 60 minutes.
2. 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.

<table>
<thead>
<tr>
<th>Item</th>
<th>Quantity</th>
<th>Storage temperature (before prep)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hoechst 33258 (20 mM solution in water)</td>
<td>5 mL</td>
<td>-20°C</td>
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</table>

3. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:
- Buffered salt solutions or media, with optimal dye binding at pH 7.4.
4. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide: www.abcam.com/assaykitguidelines

For typical data produced using the assay, please see the assay kit datasheet on our website.
5. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

5.1 Hoechst 33258 (20 mM solution in water)

Ready to use as supplied.
6. 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.

1. Pellet cells by centrifugation.
2. Resuspend the cells in buffered salt solutions or media, with optimal dye binding at pH 7.4.
3. Adherent cells in culture may be stained in situ on cover slips or in the cell culture wells.
4. Add Hoechst stain using the concentrations 0.5 - 5 µM and incubate it for 15 - 60 minutes as a guide.
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.
7. FAQs / Troubleshooting

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