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Cell Apoptosis Analysis Kit (Hoechst staining)

1 Contents

Components	HY-K1072
Fixative Solution	50 mL
Hoechst 33258 Stain	50 mL
Antifade Mounting Medium	5 mL

2 Introduction

MCE Cell Apoptosis Analysis Kit (Hoechst staining) provides a rapid and convenient method to detect cell apoptosis. Hoechst 33258, a type of blue fluorescence dye (excitation/emission maxima ≈ 352/461 nm when bound to dsDNA), stains the condensed chromatin in apoptotic cells more brightly than the chromatin in normal cells. It shows strong blue fluorescence in apoptosis cells but in normal cells it shows only weak fluorescence.

3 General Protocol

For adherent cells

1. Soak a clean coverslip in 70% ethanol for 5 minutes, and then dry it in the ultra-clean table.

Note: You can also wash the coverslip three times with sterile PBS or 0.9% NaCl, and then wash with the culture medium for later use.

2. Place the coverslip on a 6-well plate. Inoculate cells until the density reaches 50-80%.

3. Stimulate cell apoptosis. Discard the medium, suspend cells with 0.5 mL of Fixative Solution and incubate for more than 10 minutes at room temperature.

Note: It is recommended to incubate overnight at 4°C.

4. Discard the Fixative Solution and wash twice with PBS or 0.9% NaCl. 3 minutes each time.

5. Add 0.5 mL of Hoechst 33258 Stain and incubate for 5 minutes.

6. Discard the dye solution and wash twice with PBS or 0.9% NaCl. 3 minutes each time.

7. Add one drop of Antifade Mounting Medium to the slide and carefully cover the coverslip containing cells. Be careful to avoid bubbles.

8. Detect the fluorescence by fluorescence microscope.

Note: When combined with dsDNA, the maximum excitation wavelength and emission wavelength of Hoechst 33342 were 352 nm and 461 nm respectively.

For suspension cells

1. Centrifuge at 1000 g at 4°C for 3-5 minutes to collect 10^5-10^6 cells.

2. Suspend cells with 0.5 mL of Fixative Solution and incubate for more than 10 minutes at room temperature.

Note: It is recommended to incubate overnight at 4°C.

3. Centrifuge at 1000 g at 4°C for 3-5 minutes. Discard the Fixative Solution and wash twice with PBS or 0.9% NaCl. 3 minutes each time.
4. Suspend cells with 50 μL PBS or 0.9% NaCl. Drop onto the slide and dry at room temperature.
5. Add 0.5 mL of Hoechst 33258 Stain and incubate for 5 minutes.
6. Discard the dye solution and wash twice with PBS or 0.9% NaCl. 3 minutes each time.
7. Add one drop of Antifade Mounting Medium to the slide and carefully cover the coverslip. Be careful to avoid bubbles.
8. Detect the fluorescence by fluorescence microscope.

Note: When combined with dsDNA, the maximum excitation wavelength and emission wavelength of Hoechst 33342 were 352 nm and 461 nm respectively.

For tissue cells

1. Prepare embedded slice.
2. Wash twice with PBS or 0.9% NaCl. 3 minutes each time.
3. Add 0.5 mL of Hoechst 33258 Stain and incubate for 5 minutes.
4. Discard the dye solution and wash twice with PBS or 0.9% NaCl. 3 minutes each time.
5. Add one drop of Antifade Mounting Medium to the slide and carefully cover the coverslip. Be careful to avoid bubbles.
6. Detect the fluorescence by fluorescence microscope.

Note: When combined with dsDNA, the maximum excitation wavelength and emission wavelength of Hoechst 33342 were 352 nm and 461 nm respectively.

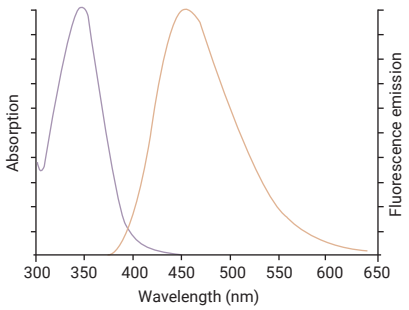

Figure Excitation/Emission spectrum of Hoechst 33258
(Left: excitation spectrum; Right: emission spectrum)

4 Storage

4°C, 6 months; -20°C, 1 year
Protect from light

5 Precautions

1. Detect the fluorescence as soon as possible to avoid fluorescence quenching.
2. Hoechst 33258 is sensitive to light, please operate away from light.
3. Hoechst 33258 is harmful, take care when handling.
4. This product is for R&D use only, not for drug, household, or other uses.
5. For your safety and health, please wear a lab coat and disposable gloves to operate.