CYDN18-CHANGYU Apoptotic Body / Hoechst Staining Kit

Catalog number	Package
CYDN1801	100 tests

Storage and shipping: store each component at the temperature indicated (Fixative at 4 °C; 100× Staining Concentrate at 4 °C protected from light; Staining Dilution Buffer at 4 °C or 15-25 °C). Valid for 6 months after receipt.

Description

In mid-to-late apoptosis, chromatin condenses and shrinks in focal areas, followed by nuclear fragmentation and the appearance of apoptotic bodies. After Hoechst staining, the DNA of nuclei or apoptotic bodies appears densely stained or as densely stained fragments. The Hoechst dye in this kit has an excitation wavelength of 350-370 nm and an emission wavelength of 465 nm; DNA emits blue fluorescence under a fluorescence microscope.

Kit components

Component	Storage	100 tests (CYDN1801)
Fixative	4 °C	50 ml
100× Staining Concentrate	4 °C, protected from light	0.5 ml
Staining Dilution Buffer	4 °C or 15-25 °C	50 ml

Notes

Note: A fluorescence microscope or laser confocal microscope capable of observing blue fluorescence is required.

Note: PBS or 0.9% NaCl solution, coverslips and slides are required.

Note: Fluorescence quenches easily; perform the operations and store the reagents protected from light as far as possible.

❖ Procedure

Adherent cells

1. Soak clean ordinary coverslips in 70% ethanol for 5 minutes or longer; dry them in a sterile clean bench or wash three times with cell-culture-grade PBS or 0.9% NaCl and once more with culture medium. Place the coverslips in a six-well plate, seed the cells and culture overnight to about 50%-80% confluence.

2. After stimulating the cells to undergo apoptosis, aspirate the culture medium completely, add 0.5 ml of Fixative and fix for 10 minutes or longer (can be left at 4 °C overnight).

The Fixative in this kit is mainly 4% paraformaldehyde. If it is not suitable for your cells or the result is poor, many fixation recipes can be used, such as a freshly prepared methanol:glacial acetic acid (3:1) fixative; choose a fixation method suited to your cells.

3. Remove the Fixative and wash twice with PBS or 0.9% NaCl for 3 minutes each time, aspirating the liquid completely. Use a shaker or agitate manually several times during washing.

4. Mix 5 μl of 100× Staining Concentrate with 0.5 ml of Staining Dilution Buffer, add it to the cells and stain for 5-10 minutes; use a shaker or agitate manually several times.

5. Wash twice with PBS or 0.9% NaCl for 3 minutes each time.

6. Place a drop of mounting medium on a slide and cover with the cell-bearing coverslip, avoiding air bubbles as far as possible. Make sure the cells contact the mounting medium and do not turn the coverslip over. Blue nuclei can be detected by fluorescence microscopy; excitation wavelength about 350 nm, emission wavelength about 460 nm.

The mounting medium can be 50% PBS/50% glycerol (mixed in equal volumes). If the fluorescence quenches too quickly for observation, use a commercial anti-fade mounting medium.

Suspension cells

1. Collect the cells by centrifugation in a 1.5 ml centrifuge tube, add 0.5 ml of Fixative, resuspend the cells gently and fix for 10 minutes or longer (can be left at 4 °C overnight).

2. Centrifuge to remove the Fixative and wash twice with PBS or 0.9% NaCl for 3 minutes each time, agitating manually during washing.

3. After the last centrifugation, aspirate most of the liquid leaving about 50 µl, resuspend the cells gently and drop them onto a slide, spreading the cells as evenly as possible.

4. Air-dry briefly so that the cells adhere to the slide and do not move with the liquid.

5. Mix 5 µl of 100× Staining Concentrate with 0.5 ml of Staining Dilution Buffer, apply evenly and stain for 5-10 minutes; remove the liquid from the edge with absorbent paper and air-dry briefly.

6. Wash twice with PBS or 0.9% NaCl for 3 minutes each time.

7. Mount and observe as for adherent cells.

Tissue sections

1. For any common section, process it to the stage at which immunostaining is normally performed, or complete routine immunostaining, and then proceed to Hoechst staining.

2. Wash twice with PBS or 0.9% NaCl for 3 minutes each time, aspirating the liquid completely. Use a shaker or agitate manually several times during washing. The operations can be carried out in a six-well plate.

3. Mix 5 µl of 100× Staining Concentrate with 0.5 ml of Staining Dilution Buffer, add it to the sections and stain for 5-10 minutes; use a shaker or agitate manually several times.

4. Wash twice with PBS or 0.9% NaCl for 3 minutes each time.

5. Mount and observe as for adherent cells.

This product is furnished for LABORATORY RESEARCH USE ONLY. Not for diagnostic or therapeutic use.