Cell Apoptosis Analysis Kit (Hoechst Method)

Cat: G3680
Size:100T
Storage: -20℃, avoid light, valid for 1 year.

Kit Components

Reagent	100T	Storage
Reagent(A): Hoechst Fixative	50mL	RT
Reagent(B): Hoechst Solution	50mL	-20℃,avoid light
Reagent(C): Fluorescence Sealer	5mL	2-8℃

Introduction

When apoptosis occurs, chromatin will shrink. After Hoechst 33258 staining, the nucleus of normal cells are normal blue, while the nucleus of apoptotic cells are dense or fragmented with some white color. Hoechst Staining Kit is often used to detect apoptosis in cultured adherent or suspension cells and tissue sections.

Hoechst Stain Kit is a rapid and simple kit for the detection of apoptosis using the classic Hoechst 33258 method. The range of cell content detected by this kit is generally 0.1-1×10^6.

Self Provided Materials

Fluorescence Microscope,PBS or Normal Saline,Slide and Coverslip,Precool 4% PFA.

Protocol(*for reference only*)

For Adherent Cells

1. Take a clean coverslip and soak it in 70% ethanol for 5min or longer, dry it in the sterile super clean table or wash it with sterile PBS or normal saline for 3 times, and then wash it with cell culture solution for 1 time. The fusion rate is about 50% - 80% when the cover glass is placed in a 6-well plate or other culture dishes and the cells are cultured overnight.
2. After the intervention conditions are added to induce apoptosis. Suck up the culture medium, add 0.5mL Hoechst fixative, and fix for 10min or longer (overnight at 4 ℃).
3. Remove the fixed liquid, wash it with PBS or normal saline twice, 3 mins each time, and suck up the liquid. It is better to use a shaker or shake it manually when washing.
4. Add 0.5mL Hoechst Solution and incubate for 5min. It is also advisable to use a shaker or shake it several times by hand.
5. Discard the dye solution, wash it twice with PBS or normal saline for 3min each time, and suck up the liquid. It is better to use a shaker or shake it manually when washing.
6. Drop a drop of Fluorescence Sealer on the slide, cover the coverslip with cells, let the cells contact the sealing agent, and try to avoid bubbles.
7. The blue nucleus can be detected by fluorescence microscope. The excitation wavelength is about 350nm and the emission wavelength is about 460nm.

For Suspension Cells

1. Collect the cell samples by centrifugation in 1.5mL centrifuge tube and discarded. Then add 0.5mL Hoechst fixative. Suspend the cells slowly and fix for 10min or longer (or overnight at 4 ℃).
2. Remove the Hoechst Fixative low-speed centrifugation and wash twice with PBS or normal saline for 3min each time. Shake manually several times when washing.
3. After centrifugation at low speed, most of the liquid is absorbed and keep the full system at about 50μl, then suspend the cells slowly and drop onto the slide to make the cell distribution as uniform as possible.
4. Slightly dry, so that the cells attach to the slide are not easy to flow with the liquid.
5. Add 0.5mL Hoechst Solution and incubate for 5min. Remove the liquid from the edge with absorbent paper and dry it slightly.
6. Discard the dye solution, wash it twice with PBS or normal saline for 3min each time, and suck up the liquid. It is better to use a shaker or shake it manually when washing.
7. Drop a drop of Fluorescence Sealer on the slide, cover the coverslip with cells, let the cells contact the sealing agent, and try to avoid bubbles.
8. The blue nucleus can be detected by fluorescence microscope. The excitation wavelength is about 350nm and the emission wavelength is about 460nm.

For Tissue Sections

1. Conventional fixation, conventional paraffin embedding. Dewaxing to distilled water before staining.
2. Wash twice with PBS or normal saline for 3min each time. Shake manually several times when washing.
3. Drop 0.5mL Hoechst Solution evenly and incubate for 5 mins.
4. Discard the dye solution, wash it twice with PBS or normal saline for 3min each time, and suck up the liquid. It is better to use a shaker or shake it manually when washing.
5. Drop a drop of Fluorescence Sealer on the slide, cover the coverslip with cells, let the cells contact the sealing agent, and try to avoid bubbles.
6. The blue nucleus can be detected by fluorescence microscope. The excitation wavelength is about 350nm and the emission wavelength is about 460nm.

Note

1. It is suggested to detect as soon as possible after staining while there are quenching problems in fluorescent dyes. The Fluorescence Sealer should also be used in dark operation.
2. In order to obtain the centrifugation of cell precipitation, for special cells, if the cell precipitation is not enough, the centrifugal force can be appropriately increased or the centrifugation time can be extended.
3. Hoechst 33258 Solution has certain irritation to human body, please pay attention to appropriate protection.
4. For your safety and health, please wear experimental clothes and disposable gloves.

Reference

[1] Yi HouYi Hou,Pengju Zhang,Dawei Wang,Jing Liu,Wei Rao*,et al.Liquid Metal Hybrid Platform-Mediated Ice–Fire Dual Noninvasive Conformable Melanoma Therapy.ACS Appl. Mater. Interfaces.May 2020.(IF 8.456)
[2] Ming-liang Zhu.Myricetin induces apoptosis and autophagy by inhibiting PI3K/Akt/mTOR signalling in human colon cancer cells.BMC Complementary Medicine and Therapies.July 2020.(IF 1.979)
[3] Min Gan,Hongbiao Ding,Gang Chen,et al.6-Formylindolo[3,2-b]carbazole reduces apoptosis induced by benzo[a]pyrene in a mitochondrial-dependent manner.Cell Biology International.August 2020.(IF 2.571)