

Apoptotic body/hoeschst Stain Kit

Product Number: DNK1801

Components

Component	Storage	DNK1801 100 Preps
Buffer SL	4°C	50 ml
100×Buffer DC	4 °C, avoid light	0.5 ml
Buffer DD	4°C or RT	50 ml

Description

The morphological changes of cells in the middle and late stages of apoptosis are chromatin aggregation and consolidation in local areas, followed by nuclear fragmentation and the appearance of apoptotic bodies. Under Hoeschst staining, the DNA of the nucleus or apoptotic bodies will exhibit dense staining or fragmented dense staining. The excitation wavelength of Hoechst dye ultraviolet light in this reagent kit is 350-370nm; The emission wavelength is 465nm, and DNA emits blue fluorescence under a fluorescence microscope.

Note

1. A fluorescence microscope or laser confocal microscope capable of observing blue fluorescence is required.
2. PBS or 0.9% NaCl solution, cover glass and slide are required.
3. Fluorescence is easily quenched, so it should be handled and stored away from light as much as possible.

Protocol(Please read the note before the experiment)

1. Adherent cells

- 1.1. Soak a regular clean cover glass in 70% ethanol for 5 minutes or longer, blow dry in a sterile ultra clean bench, or wash three times with cell culture grade PBS or 0.9% NaCl solution, and then wash once with cell culture medium. Place the cover glass in a six well plate and plant it into the cell culture overnight, until it is about 50% -80% full.
- 1.2. After stimulating cell apoptosis, aspirate the culture medium thoroughly, add 0.5ml Buffer SL, and fix for 10 minutes or longer (overnight at 4°C).

The buffer SL used in this kit is mainly 4% paraformaldehyde. If it is not suitable for your cells or the effect is not good, many fixed formulas such as "methanol: glacial acetic acid (3:1) ready to use" can be used, and suitable fixation methods can be selected according to your own cell characteristics.

- 1.3. Remove Buffer SL, wash twice with PBS or 0.9% NaCl for 3 minutes each time, and absorb all the liquid. When washing, it is advisable to use a shaker or manually shake it several times.
- 1.4. Mix 5µl 100×Buffer DC with 0.5ml of Buffer DD and add staining for 5-10 minutes. It is also advisable to use a shaker or manually shake several times.
- 1.5. Drop a drop of sealing solution onto a glass slide using PBS, cover it or wash it twice with 0.9% NaCl for 3 minutes each time.
- 1.6. Cover glass with cells attached, try to avoid bubbles as much as possible. Contact the cells with the sealing solution and do not reverse it. Fluorescence microscopy can detect blue nuclei. The excitation wavelength is around 350nm, and the emission wavelength is around 460nm.

The sealing solution can use 50% PBS/50% glycerol (mixed by equal volume). If the fluorescence quenching is too fast and affects observation, commercial anti fluorescence quenching sealing solutions should be selected.

2. Suspended cells

- 2.1. Centrifuge the collected cell samples into a 1.5ml centrifuge tube, add 0.5ml Buffer SL, slowly suspend the cells, and fix

for 10 minutes or longer (overnight at 4 °C).

- 2.2. Centrifuge buffer SL and wash twice with PBS or 0.9% NaCl for 3 minutes each time. Manually shake during washing.
- 2.3. After the last centrifugation, absorb most of the liquid and retain about 50µl of the liquid. Then slowly suspend the cells and add them dropwise onto a glass slide, trying to distribute the cells evenly.
- 2.4. Let it dry slightly to make the cells adhere to the glass slide and not easily flow with the liquid.
- 2.5. Mix 5µl 100×Buffer DC with 0.5ml of Buffer DD and stain evenly for 5-10 minutes. Use absorbent paper to remove the liquid from the edge and let it dry slightly.
- 2.6. Wash twice with PBS or 0.9% NaCl for 3 minutes each time.
- 2.7. Seal and observe like adherent cells.

3. Tissue slicing

- 3.1. For any common slice, it can be processed to the point where routine immunostaining can be performed, or subsequent Hoeschst staining can be performed after completing routine immunostaining.
- 3.2. Wash twice with PBS or 0.9% NaCl for 3 minutes each time, and absorb all the liquid. When washing, it is advisable to use a shaker or manually shake it several times. Can be operated in a six hole plate.
- 3.3. Mix 5µl 100×Buffer DC with 0.5ml of Buffer DD and add staining for 5-10 minutes. It is also advisable to use a shaker or manually shake several times.
- 3.4. Wash twice with PBS or 0.9% NaCl for 3 minutes each time
- 3.5. Seal and observe like adherent cells.