V02

细胞凋亡-Hoechst 染色试剂盒

货号：G3680
规格：100T
保存：-20℃，避光保存，有效期 1 年。

产品组成：

名称	100T	保存
试剂(A): Hoechst固定液	50mL	室温
试剂(B): Hoechst染色液	50mL	-20℃，避光
试剂(C): 荧光封片剂	5mL	2-8℃

产品介绍：

当细胞发生凋亡时，染色质会固缩，Hoechst33258 染色后在荧光显微镜下观察，正常细胞的细胞核呈正常的蓝色，而凋亡细胞的细胞核会呈致密浓染，或呈碎块状致密浓染，颜色有些发白。细胞凋亡-Hoechst 染色试剂盒经常用于培养的贴壁或悬浮细胞以及组织切片的细胞凋亡检测。

细胞凋亡-Hoechst 染色试剂盒是一种采用经典的 Hoechst33258 进行细胞凋亡检测的快速简便的试剂盒。该试剂盒检测细胞含量范围在 0.1～1×10^6 之间。

自备材料：

荧光显微镜、PBS或生理盐水、载玻片、盖玻片、4%组织细胞固定液

操作步骤：(*仅供参考*)

(一)贴壁细胞

1、取洁净盖玻片在70%乙醇中浸泡5min或更长时间，无菌超净台内吹干或用无菌的PBS或生理盐水洗涤3次，再用细胞培养液洗涤1次。将盖玻片置于6孔板或其他培养皿内，接种细胞培养过夜，使融合率约为50%～80%。
2、加入干预条件使细胞发生凋亡后，吸尽培养液，加入Hoechst固定液0.5mL，固定10min或更长时间(可4℃过夜)。
3、去除固定液，用PBS或生理盐水洗2次，每次3min，吸尽液体。洗涤时宜用摇床，或手动晃动。
4、加入Hoechst 33258染色液0.5mL，孵育5min。也宜用摇床，或手动晃动数次。
5、弃染色液，用PBS或生理盐水洗2次，每次3min，吸尽液体。洗涤时宜用摇床，或手动晃动。
6、滴一滴抗荧封片剂于载玻片上，盖上贴有细胞的盖玻片，让细胞接触封片剂，尽量避免气泡。
7、荧光显微镜可检测到呈蓝色的细胞核。激发波长350nm左右，发射波长460nm左右。

(二)悬浮细胞

1、离心收集细胞样品于1.5mL离心管内并弃液，加入Hoechst固定液0.5mL，缓缓悬起细胞，固定10min或更长时间(亦可4℃过夜)。
2、低速离心去除固定液，用PBS或生理盐水洗2次，每次3min。洗涤时手动晃动数次。
3、低速离心离心后吸去大部分液体保留约50μl液体，再缓缓悬起细胞，滴加至载玻片上，尽量使细胞分布均匀。
4、稍晾干，使细胞贴在载玻片上不易随液体流动。
5、滴加Hoechst 33258染色液0.5mL，孵育5min。用吸水纸从边缘吸去液体，微晾干。
6、弃染色液，用PBS或生理盐水洗2次，每次3min，吸尽液体。洗涤时宜用摇床，或手动晃动。
7、滴一滴抗荧光封片剂于载玻片上，盖上一洁净的盖玻片，尽量避免气泡。
8、荧光显微镜可检测到呈蓝色的细胞核。激发波长350nm左右，发射波长460nm左右。

(三)组织切片

1、常规包埋切片。
2、用PBS或生理盐水洗2次，每次3min。洗涤时手动晃动数次。
3、均匀滴上Hoechst 33258染色液0.5mL，孵育5分钟。

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4、 弃染色液，用PBS或生理盐水洗2次，每次3min，吸尽液体。洗涤时宜用摇床，或手动晃动。
5、 将切片置于载玻片上，滴一滴抗荧光封片剂，盖上一洁净的盖玻片，尽量避免气泡。
6、 荧光显微镜可检测到呈蓝色的细胞核。激发波长350nm左右，发射波长460nm左右。

注意事项：

1、 荧光染料都存在淬灭的问题，建议染色后尽快检测。使用抗荧封片剂时也应避光操作。
2、 在为了获得细胞沉淀的离心的过程中，对于特殊细胞，如果细胞沉淀不充分，可以适当提高离心力或延长离心时间。
3、 Hoechst 33258染色液对人体有一定刺激性，请注意适当防护。
4、 为了您的安全和健康，请穿实验服并戴一次性手套操作。

参考文献：

[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)

V02

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

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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)