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Selectively Apoptotic DNA Ladder Extract Kit

Product Number: DNK1701

Shipping and Storage

To maintain activity and facilitate transportation, Enzyme B customers receive freeze-dried powder. After receiving it, add 500μl of sterilized water (25 times) or 1m of sterilized water (50 times) to dissolve and store at -20°C. Enzyme A and Enzyme B are enzyme solutions, and repeated freeze-thaw cycles should be avoided to reduce activity. If they need to be used multiple times, it is best to pack them according to the amount used each time and store them at -20°C.

Components

Component	Storage	DNK1701 25 Preps	DNK1702 50 Preps
Extraction Buffer	4°C	5 ml	10 ml
10% SDS	RT	500μl	1 ml
Enzyme A	-20°C	500μl	1 ml
Enzyme B	-20°C	500μ1	1 ml
Precipitant	4°C	3.5 ml	7 ml

Description

A significant morphological feature of apoptotic or programmed death cells is that the chromosome DNA breaks regularly with the nucleosome as a unit (185bp) to form a DNA fragment with a length of about n × 185bp(n=1,2,3,4...). The agarose gel electrophoresis shows a ladder like apoptotic DNA Ladder, which is the most intuitive feature of apoptotic cells. This reagent kit selectively separates and extracts apoptotic DNA layers from tissues and cells. By selectively separating genomic DNA from apoptotic DNA layers, it minimizes the observation interference of genomic DNA on apoptotic DNA layers, significantly improving detection sensitivity. The reaction can be carried out in a microcentrifuge tube, completed in 2.5 hours, which is fast and convenient; No organic extraction is required, the detection sensitivity is extremely high, and DNA ladder can be detected from approximately 2000 apoptotic cells.The recommended starting cell count is $5\sim10\times10^5$, but the input cell count can vary between $1\times10^5\sim5\times10^6$. The principle is that the total cell should contain at least $1\sim2\times10^4$ apoptotic cells. More than 2×10^4 apoptotic cells can usually obtain very clear apoptotic DNA layers. This kit can also be used to extract apoptotic DNA ladder from tissues. However, compared with cultured cells, the poor regularity of the time, location, and degree of apoptotic cells in overall animal tissue often makes it difficult to accurately obtain samples, which may significantly affect the experimental results. But as long as the organization does experience apoptosis, experienced users can also use this kit to extract apoptotic DNA ladder from the organization.

Features

1. Excessive ethidium bromide staining will reduce the sensitivity of DNA strip detection, and the gel can be washed with water for 10~30 minutes. If washed too much, it can be re dyed with ethidium bromide. A more sensitive DNA staining agent SYBR Green can be used. Acrylamide DNA gel electrophoresis and DNA silver staining can also be performed.
2. After intervention treatment of cells, apoptosis may only be most pronounced at a certain point in time or at a certain intervention intensity. Pre experiments are needed to determine the optimal intervention time or intensity. At this time, Apoptotic body/hoeschst Stain Kit (DNK1801) can also be used for rapid staining of apoptotic bodies for observation.
3. The recommended starting cell count is $5\sim10\times10^5$, but the input cell count can vary between $1\times10^5\sim5\times10^6$. The principle is that the total cell should contain at least about $1\sim2\times10^4$ apoptotic cells. More than 2×10^4 apoptotic cells can usually obtain very clear apoptotic DNA layers. A well in a six well plate is equivalent to a 35 mm culture dish that can produce $1\sim10\times10^5$ cells when fully grown. If the incidence of cell apoptosis is 10%, approximately $1\sim10\times10^4$ apoptotic cells can be obtained after treatment, which should be sufficient to obtain clear apoptotic DNA layers. On the contrary, if clear apoptotic DNA ladder

cannot be obtained from>36 cells, it indicates that the number of apoptotic cells is less than 1%. At this point, increasing the amount of cells is also difficult to achieve.

4. Extract apoptotic DNA ladder from tissue blocks. Take 10-20 mg tissue blocks and place them in a small glass homogenizer. Add 100-200μl Extraction Buffer and manually homogenize 15-20 times. Take out the homogenate and let it sit on ice for 5-10 minutes. Oscillate for 10 seconds. Collect the supernatant at 4500rpm for 10 minutes and transfer it to a new 1.5ml centrifuge tube to perform extraction step 3. Another method is to cut 30-50mg of tissue and homogenize it in PBS to make a cell suspension. Centrifuge the collected cells and continue with step 2 for extraction.
5. High quality agarose was used to make thin agarose gel (about 2-4 mm thick) by using a sample comb with smaller width and narrower thickness; Using a lower voltage for slow electrophoresis will significantly increase the sensitivity of detecting apoptotic DNA bands. The electrophoresis distance should not be too long, otherwise it will cause small apoptotic DNA bands to diffuse and reduce resolution.

Protocol

1. Rinse the cells twice with PBS and collect 5~10×10^5 cells using a micro centrifuge at 500×g 4°C for 5 minutes (preferably as a control for non apoptotic cells). Be careful to use a pipette to aspirate and discard the supernatant, even if there is liquid adhering to the wall.
2. After gently scattering the cell precipitates at the bottom of the centrifuge tube with your fingers, add 100μl of Extraction Buffer and mix vigorously with an oscillator for 10 seconds. Centrifuge 1,100~1,600×g (approximately 3500-4500rpm) for 5 minutes.
3. Do not touch the sediment at the bottom of the tube, transfer the supernatant to a new 1.5ml centrifuge tube.
4. Repeat step 2 of the precipitation process again.
5. Combine the supernatant with the supernatant from operation step 3, totaling about 200μl, as a crude extraction solution (containing apoptotic DNA fragments, non apoptotic chromosome DNA has been removed by precipitation).
6. Add 20μl of 10% SDS solution to the crude extract, then add 20μl of Enzyme A, mix well, and incubate at 56℃ for 1 hour.
7. Add 20μl of Enzyme B to the above mixture, mix well, and incubate at 37℃ for 1 hour, or until it becomes transparent (overnight is acceptable).
8. Add Precipitant 130μl to the above mixture, invert and mix well, then add 1ml of ethanol, mix well, and let it stand at -20℃ for more than 1 hour (precipitate and kill DNA fragments)
9. Centrifuge at least 13000rpm at 4°C for 15 minutes, discard the supernatant, rinse with 1ml of 70% ethanol, centrifuge, pour out ethanol, and try to remove any liquid adhering to the tube wall. Open the pipe opening and air dry the sediment at room temperature.
10. Use 17μl double distilled water or TE Buffer to fully dissolve and precipitate, add 3μl 6×DNA gel loading Buffer, shake and mix. Take all 20μl samples or an appropriate amount of samples for 1% agarose gel electrophoresis. Dyeing with ethidium bromide and taking photos under UV observation (when the incidence of apoptosis is low, adding excessive TE Buffer to dissolve the precipitate may lead to a concentration that is too thin to detect DNA Ladder, so the dosage can be reduced, and vice versa, it can be increased).