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Apoptotic DNA Ladder Fast Kit

Product Number: DNK1601

Shipping and Storage

1. When the ambient temperature is low, some detergent ingredients in Buffer YL will precipitate and become turbid or precipitate. It can be heated in a 37℃ water bath for a few minutes and gently shaken to restore clarity. Do not shake violently to avoid excessive foam formation.
2. Buffer PP may experience precipitation and precipitation, and can be re dissolved by taking a water bath at 37℃ for a few minutes. If it cannot be completely dissolved, it will not affect the effectiveness of use. Simply take the upper solution.
3. To avoid volatilization, oxidation, and pH changes caused by prolonged exposure of reagents to the air, each solution should be covered tightly in a timely manner after use.

Components

Component	Storage	DNK1601 20preps	DNK1602 50preps
Buffer CB	RT	6 ml	15 ml
Buffer WB	RT	6 ml	15 ml
Buffer EB	RT	15ml	15 ml
Adsorption column AC	RT	20	50
Collection tube (2ml)	RT	20	50

Description

When cells undergo apoptosis, chromatin DNA breaks between nucleosomes, ultimately forming DNA fragments with integer multiples of 200bp. These DNA fragments are extracted, and after electrophoresis and ethidium bromide staining, they form a ladder like appearance, known as DNA Ladder. After the lysis of blood and tissue cultured cells in Buffer CB, the released DNA fragments selectively adsorb onto the silica matrix membrane in a highly dissociated salt state. Then, through a series of rapid rinsing centrifugation steps, impurities such as salt, cell metabolites, and proteins are removed. The lowest salt buffer EB washes the DNA Ladder fragments off the silica matrix membrane.

Features

1. No toxic reagents such as phenol are required, and no steps such as ethanol precipitation are required.
2. Our company's unique lysis/binding solution formula effectively lyses cells, and using this reagent kit does not require expensive protease K treatment, greatly reducing usage costs and accelerating processing speed.
3. Time saving, concise, and single sample operation can generally be completed within 10 minutes.

Application

Suitable for rapid extraction of apoptotic DNA Ladder

Note

1. All centrifugation steps are completed at room temperature using a traditional desktop centrifuge with a speed of up to 13000rpm.
2. Preheat the required water bath to 70℃ for later use before starting the experiment.
3. Buffer CB contains irritating compounds, and latex gloves should be worn during operation to avoid contact with skin, eyes, and clothing. If it gets on the skin or eyes, rinse with plenty of water or physiological saline.
4. Generally, the DNA production of 2×10^6 cultured cells is 10-20μg, and the typical production of 200μl whole blood is 3-6μg.

5. In general, the typical sample size for electrophoresis detection is 2-3μg of purified DNA. If the apoptosis rate is low, it is possible to only see genomic DNA and not DNA layers. You can try increasing the sample size.

Protocol(Please read the note before the experiment)

Tip: Before the first use, please add the specified amount of anhydrous ethanol to Buffer WB and mix well. After adding, please mark the box with a check mark indicating that ethanol has been added in a timely manner to avoid adding it multiple times!

1. Take 2×10^6 cells (suspended or tissue cultured cells resuspend in 200μl PBS) or 200μl whole blood (containing approximately 2×10^6 cells) and add 200μl Buffer CB. Immediately vortex and shake thoroughly to mix well.
2. **Optional steps:** If there is a significant amount of RNA residue that affects the observation of apoptotic DNA layers, you can add 20μl of RNase A (25mg/ml) solution before adding 200μl of Buffer CB, shake well, and let it stand at room temperature for 5-10 minutes.

   The maximum starting amount for cell or whole blood processing can reach 300μl. If the starting amount is between 200μl-300μl, it is necessary to increase the amount of reagents used proportionally.
3. Leave at room temperature (15℃ -20℃) for 10 minutes.
4. Add 100μl of isopropanol, immediately vortex and shake thoroughly, and flocculent precipitation may occur at this time.

   Adequate mixing with appropriate force is crucial in the above steps, as insufficient mixing can significantly reduce yield. If the sample is viscous and difficult to mix, vortex oscillation can be used for 15 seconds.
5. Add the previous mixture (including possible precipitates) to an adsorption column AC, centrifuge at 13000rpm for 30 seconds (with the adsorption column placed in the collection tube), and discard the waste liquid in the collection tube.
6. Add 700μl of Buffer WB (please check if anhydrous ethanol has been added first!), centrifuge at 12.000rpm for 30 seconds, and discard the waste liquid.
7. Add 500μl of Buffer WB, centrifuge at 12000rpm for 30 seconds, and discard the waste liquid.
8. Put the adsorption column AC back into the empty collection tube, centrifuge at 13.000rpm for 2 minutes, and try to remove Buffer WB as much as possible to avoid residual ethanol in Buffer WB affecting the elution efficiency and downstream reaction.
9. Take out the adsorption column AC and place it in a clean centrifuge tube. Add 100μl of Buffer EB (pre heated in a 65-70℃ water bath) to the middle of the adsorption membrane. Let it stand at room temperature for 3-5 minutes and centrifuge at 12000 rpm for 1 minute. Add the obtained solution back into the centrifugal adsorption column, let it stand at room temperature for 2 minutes, and centrifuge at 12.000 rpm for 1 minute.

   The larger the elution volume, the higher the elution efficiency. If a higher DNA concentration is required, the elution volume can be appropriately reduced, but the minimum volume should not be less than 50μl. If the volume is too small, it will reduce the DNA elution efficiency and DNA production.
10. DNA can be used directly or stored at -20℃, but not for more than 14 days.
11. Take approximately 2-3μg of purified DNA for electrophoresis detection (note that the typical yield of 200l human whole blood is only 3-6μg).