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Apoptosis DNA Ladder Extraction Kit With Spin Column

Cat #: KTA1101 Size: 50 T/100 T

	Apoptosis DNA Ladder Extraction Kit With Spin Column		
REF	**Cat #:** KTA1101	LOT	**Lot #:** Refer to product label
	Applicable samples: Animal Tissues, Cells		
	Storage: Store according to the recommended storage conditions of each component, stable for 12 months		

Assay Principle

Cellular endonucleases are activated and cut genomic DNA between nucleosomes into 180-200bp DNA fragments in cells undergoing apoptosis. When DNA is extracted for electrophoresis detection during cell apoptosis, DNA Ladder of 180-200 bp or its integral multiple can be found. DNA Ladder is a hallmark event in cells undergoing apoptosis, usually observed DNA ladder, cell apoptosis can be determined. Apoptotic DNA Ladder Extraction Kit With Spin Column uses DNA Spin Column to extract DNA Ladder, which is more convenient than traditional phenol chloroform extraction method for alcohol precipitation, and small DNA fragments are less likely to be lost. It is very efficient to extract small DNA Ladder down to180-200 bp in length, at the same time, genomic DNA up to 50 kb is also extracted.

Materials Supplied and Storage Conditions

Kit components		Size		Storage conditions
		50 T	100 T	
Apoptotic DNA Ladder Extraction Kit with Spin Column (Part 1 of 2)	Lysis Buffer A	12 mL	24 mL	RT
	Lysis Buffer B	12 mL	24 mL	RT
	Wash Buffer A	21 mL	42 mL	RT
	Wash Buffer B	8 mL	16 mL	RT
	Elution Buffer	10 mL	20 mL	RT
	Spin Column	50	50×2	RT
	Collection Tube	50	50×2	RT
Apoptotic DNA Ladder Extraction Kit with Spin Column (Part 2 of 2)	RNase A	110 µL	220 µL	-20℃
	Proteinase K	550 µL	1.1 mL	-20℃

Materials Required but Not Supplied

- Precision pipettes, disposable pipette tips, centrifuge tube
- Centrifuge, vortex mixer
- Water bath, gel electrophoresis apparatus
- Ethanol anhydrous, PBS
- Agarose, TAE, Loading Buffer, DNA Marker, Ethidium bromide

Reagent Preparation

Lysis Buffer A: Ready to use as supplied. Store at RT.

Lysis Buffer B: Ready to use as supplied. Store at RT.

Wash Buffer A: At the first use, 50 T add 9 mL of Ethanol anhydrous to Wash Buffer A, 100 T add 18 mL of Ethanol anhydrous to Wash Buffer A,mix well and mark on the bottle. Store at RT.

Wash Buffer B: At the first use, 50 T add 32 mL of Ethanol anhydrous to Wash Buffer B, 100 T add 64 mL of Ethanol anhydrous to Wash Buffer B, mix well and mark on the bottle. Store at RT.

Elution Buffer: Ready to use as supplied. Store at RT.

RNase A: Ready to use as supplied. Store at -20℃.

Proteinase K: Ready to use as supplied. Store at -20℃.

Assay Procedure

Note: DNA extraction requires a 56℃ or 70℃ water bath. Please make water bath ready in advance.

A. For cells

1. Collect 1×10^6 cells, resuspend cells in 200 µL of PBS by pipetting or flicking the tube gently.

Note: For frozen cell precipitation, thaw the cell precipitation and gently flick the tube, then add PBS to resuspend cells.

2. Add 2 µL RNase A and mix well by gentle vortexing. Incubate at room temperature for 3-5 min.
3. Add 10 µL Proteinase K and mix well by gentle vortexing.
4. Add 200 µL Lysis Buffer B, mix immediately by gentle vortexing and incubate at 70℃ for 10 min.

Note: After adding Lysis Buffer B, it must be vortexed and mixed immediately. Proteinase K should not be mixed directly with Lysis Buffer B.

5. Add 200 µL Ethanol anhydrous and mix thoroughly by gentle vortexing.

Note: Precipitates may be visible after addition of ethanol, which is normal.

6. Transfer all the content from step.5, including any precipitates that may have formed, to a Spin Column placed in a Collection Tube. Centrifuge at 12,000 rpm for 1 min. Discard the flow through. Reuse the Collection Tube.

Note: Before performing this step, place the Spin Column on the Collection Tube, reuse the Collection Tube after discard the liquid waste. All the contents from step.5 must be transferred into the Spin Column. Otherwise, the DNA extraction result will be severely compromised.

7. Add 0.5 mL of Wash Buffer A to the Spin Column (Check whether Ethanol anhydrous has been added before use), and centrifuge at 12,000 rpm for 1 min. Discard the flow through. Reuse the Collection Tube.
8. Add 0.6 mL of Wash Buffer B to the Spin Column (Check whether Ethanol anhydrous has been added before use), and centrifuge at 12,000 rpm for 1 min. Discard the flow through. Reuse the Collection Tube.
9. Centrifuge at 12,000rpm for another 2 min to remove residual ethanol.
10. Place the Spin Column in a new 1.5 mL centrifuge tube, open the lid and let it dry at room temperature for 3-5 min. Add 50-100 µL of Elution Buffer directly to the Spin Column membrane, and sit at room temperature for 1-3 min. Centrifuge at 12,000rpm for 1-2 min to elute the DNA.

Note: To increase the amount of DNA extraction, the Elution Buffer can be preheated to 56℃ before elution, or the solution obtained from the first elution can be re added to the Spin Column for elution.

11. Load samples onto a 1% agarose gel for electrophoresis.

Note: Be sure to use freshly prepared electrophoresis solutions. In order to get a better DNA separation result,

electrophoresis should be performed with a longer gel and a lower voltage. Using thinner comb gel often results in better ladder electrophoresis results.

B. For animal tissues

Note: Compared with cultured cells, the location and regularity of apoptotic cells in animal tissue samples are uncertain, and differences in sampling location. The difference of sampling location and amount will significantly affect the extraction effect of the final DNA Ladder. It is recommended that experienced users use this kit to extract tissue apoptotic DNA Ladder.

1. Cut 25 mg tissue sample into pieces as small as possible, and add 190 µL Lysis Buffer A.

Note: Use no more than 25 mg tissues generally, and no more 10 mg for spleen tissues. Otherwise, tissues could not be lysed sufficiently. Smaller tissue fragments make lysis faster and more effective. Both fresh and frozen tissues can be used.

2. Add 10 µL Proteinase K. Mix well by gentle vortex and incubate at 56℃ in a water bath until tissues are completely lysed.

Note: Generally, the incubation time requires 1-3 h, but it varies for different tissues. For convenience, overnight incubation can be performed, without any negative effect on DNA extractions. Tissue lysate may be viscous after complete lysis, but should not be gelatinous to block the membrane of DNA Purification Column. Tissue lysate is gelatinous even after overnight digestion, indicating too much tissues are used and the overall reaction system should be scaled up appropriately. The tissue sample can also be ground into powder by liquid nitrogen to shorten the lysis time.

3. Add 2 µL RNase A. Mix well by gentle vortex and incubate at room temperature for 3-5 min.
4. Vortex vigorously for 15 s, then add 200 µL Lysis Buffer B and mix immediately by vortex. Incubate at 70℃ for 10 min.

Note: White precipitates may be visible after adding Lysis Buffer B, but will be redissolved in most cases during the incubation at 70℃. The presence of undissolved precipitates does not affect subsequent procedures. Upon the addition of Lysis Buffer B, gelatinous structures may also be formed in the lysate of some tissues, such as lungs and spleen, which should be disrupted as much as possible by vigorous shaking or vortex.

5. The following steps are the same as the steps of A.5-A.11.

Precautions

1. Precipitation may occur in Lysis Buffer A or Lysis Buffer B at low temperatures, which is a normal phenomenon. If there is precipitation, incubate in a 56℃ water bath to dissolve the precipitation, mix well and use.
2. All procedures including centrifugation are performed at room temperature, unless otherwise stated.
3. The Collection Tube should be reused several times for a single extraction. Do not discard it in the middle of extraction.

Recommended Products

Catalog No.	Product Name
KTP3007	ExKine™ Pro Total Protein Extraction Kit for Animal Cultured Cells/Tissues
KTA2010	One-step TUNEL Apoptosis Assay Kit (Green Fluorescence)
KTA2011	One-step TUNEL Apoptosis Assay Kit (Orange Fluorescence)
KTA0002	Annexin V-AbFluor™ 488/PI Apoptosis Detection kit
KTA4001	Mitochondrial Membrane Potential Assay Kit (JC-1)

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.