Apoptotic Cell DNA Laddering Kit

For detection of double-stranded DNA breaks in apoptotic cells

Catalog No. 30-1231, 20 tests

Instructions

Store at 15 to 30°C.

For laboratory research use only. Not for human, clinical, or diagnostic use.

American Type Culture Collection
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Manassas, VA 20108 USA
www.atcc.org

800-638-6597 or 703-365-2700
Fax: 703-365-2750
E-mail: tech@atcc.org
Or contact your local distributor.

TABLE OF CONTENTS

INTRODUCTION

Background

Degradation of chromosomal DNA is a hallmark of apoptosis. Late in the apoptotic process, caspase-activated endogenous endonucleases cleave chromosomal DNA between the nucleosomes, generating a series of DNA fragments with multiples of 180 to 220 bases (1-3). These fragments form a ladder when the extracted DNA is separated by gel electrophoresis and stained by ethidium bromide.

ATCC's Apoptotic Cell DNA Laddering Kit contains reagents needed to extract DNA fragments from cells and analyze the products by horizontal gel electrophoresis.

References

1. Rosl F. Nucleic Acids Res. 1992. 20: 5243, 1992.
2. Smith ML and Fornace Jr. AJ. Mutat. Res. 340: 109-124, 1996.
3. Nagata S. Exp. Cell Res. 256: 12-18, 2000.

Precautions

- Some components, such as the lysis and extraction solutions, are harmful if ingested or inhaled and may be irritating to eyes, respiratory system, and skin. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Check the MSDS for further information on components of the kit.

- Exercise caution and wear gloves, lab coats, and eye protection while using kit components. Gloves are required when handling solutions or gels containing ethidium bromide. Protective goggles and clothing are necessary during exposure to UV light.

- Discard organic and hazardous materials following standard laboratory procedures.

KIT COMPONENTS

Store all reagents at 15 to 30°C.
If phase separation occurs with Lysis Solution, heat to 50°C for 5 minutes and cool to room temperature prior to use.

Reagent	Quantity
Sample Buffer	2 ml
Lysis Solution	2 x 1 ml
Extraction Solution	20 ml
Extraction Buffer	8 ml
Sodium Acetate	1 ml
DNase-Free Water (contains RNase A)	2 ml
5x Gel Loading Buffer	250 µl
Electrophoresis Gel Powder	6 g

MATERIALS REQUIRED BUT NOT SUPPLIED

Micropipettors and tips
Microcentrifuge
Vortex
Horizontal gel electrophoresis apparatus and power supply
UV transilluminator
UV spectrophotometer
2-ml microcentrifuge tubes
Gloves
2-propanol (isopropanol)
70% ethanol in dH_2O
Chloroform (optional)
TAE buffer (See appendix for formula)
Ethidium bromide solution (0.5 mg/ml in dH_2O)
Deionized water
Dulbecco's Phosphate Buffered Saline (PBS) without calcium or magnesium (ATCC catalog no. 30-220)

PROTOCOL

Experimental Outline

Chromosomal DNA is isolated from cells and then separated by gel electrophoresis. When the DNA is stained with ethidium bromide (not included), DNA from apoptotic cells will display a ladder of sizes with multiples of 180 to 220 bp. For best results, the process should be completed without stopping and the samples processed immediately. If necessary, the samples may be stored overnight at –20°C before final processing of the isolated DNA at step 11.

Isolation Of DNA From Cells In Culture

Step	Action	
1	**Suspension Cells**	**Adherent Cells (75-cm^2 flask)**
	Count cells and harvest 10^5 to 10^7 cells by centrifuging at 300 x g for 10 minutes.	Decant medium and wash the monolayer with cold (2 to 8°C) PBS.
2	Resuspend the cells in 1 ml of cold (2 to 8°C) PBS and transfer to a 2-ml microcentrifuge tube.	Lyse the cells directly in the flask by adding 100 µl of Sample Buffer followed by 100 µl of Lysis Solution.
3	Pellet the cells by centrifugation and resuspend the cell pellet in 100 µl of room temperature Sample Buffer (10^6 to10^8 cells/ml).	Gently scrape and pipette the cells until they are completely lysed (5 to 10 minutes).
4	Incubate the cell solution at room temperature for 5 to 15 minutes.	Transfer the sample to a 2-ml microcentrifuge tube.
5	Lyse the cells by adding 100 µl of Lysis Solution. Mix thoroughly by inverting the tube.	–
6	Shake the **Extraction Solution** bottle and then add 700 µl to each sample.	
7	Add 400 µl of **Extraction Buffer** to each sample and vortex for 10 seconds.	
8	Centrifuge at 12,000 x g for 5 minutes in a microcentrifuge at room temperature.	

9	Transfer the upper layer (aqueous phase) to a new 2-ml microcentrifuge tube. **Note:** Avoid removing the organic and interphase layers. If the transferred aqueous phase is cloudy, a chloroform extraction may be required. See the troubleshooting section on page 8 for details.
10	Add 0.1 volume (40 µl) of sodium acetate to the aqueous phase (DNA sample) and mix by inverting the tube.
11	Add an equal volume (440 µl) of 2-propanol and mix by inverting. **Note:** Optional stopping point. Samples may be stored at –20°C overnight.
12	Centrifuge at 12,000 x g for 10 minutes at room temperature.
13	Carefully remove the supernatant without disturbing the pellet. Add 1 ml of 70% ethanol to the pellet. Mix gently by inverting the tube several times.
14	Centrifuge at 12,000 x g for 5 minutes at room temperature.
15	Carefully remove the supernatant without disturbing the pellet. Allow pellet to dry with the aid of a vacuum centrifuge apparatus or invert the tube on a laboratory tissue and allow the liquid to evaporate.
16	Resuspend the pellet in 100 µl of DNase-Free Water. (This water contains RNase A.)
17	Determine the concentration of the DNA by measuring the absorbance at 260 nm (see appendix, p. 6).
18	Adjust the DNA concentration to 1 µg/µl with DNase-Free Water.
19	Analyze by gel electrophoresis or store the DNA sample at 2 to 8°C.

Gel Electrophoresis

Step	Action
1	Prepare a 1.5% horizontal electrophoresis gel in TAE buffer (see appendix, p. 7).
2	Dilute 1 µl of each DNA sample with 8 µl of DNase-Free Water.
3	Add 2 µl of 5x Gel Loading Buffer to each sample and load into the wells of the gel.
4	Separate the DNA fragments by electrophoresis until the bromophenol blue tracking dye reaches the lower third of the gel.
5	Stain the gel with 0.5 µg/ml ethidium bromide prepared in dH_2O or TAE Buffer for 15 minutes.
6	Briefly destain the gel in dH_2O or TAE Buffer.
7	Visualize the DNA using a UV transilluminator.

DATA INTERPRETATION

In the majority of cell types, a typical ladder pattern of bands in multiples of approximately 200 base pairs is indicative of apoptosis (Figure 1). Although the method is not quantitative, qualitative comparisons can be made with samples prepared from an equivalent number of nonapoptotic control cells. DNA from nonapoptotic cells have a higher molecular weight. Viable cell populations may contain a minor, but measurable, number of apoptotic cells whose DNA may reveal a faint laddering pattern.

NOTE: The appearance of two distinct bands may indicate the presence of RNA contamination. Refer to the troubleshooting section on page 9 for information on removing RNA.

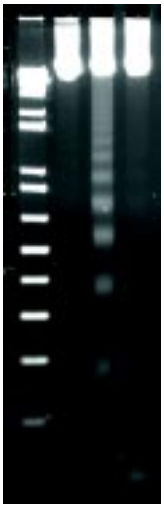

Figure 1. Gel electrophoresis of DNA isolated from 10^6 control and induced WEHI 7.1 cells (ATCC TIB-53). Lane 1: 1 kb ladder. Lane 2: DMSO-treated control cells. Lane 3: staurosporine-induced cells. Lane 4: control cells. Induced cells were treated for 30 minutes with 1µM staurosporine and allowed to grow overnight in fresh medium.

APPENDIX

DNA Quantitation

Step	Action
1	Add 5 µl of your DNA sample to 1 ml of dH_2O.
2	Read the optical density at 260 nm of your sample using a UV spectrophotometer against a water blank.
3	Determine the DNA concentration of the undiluted sample by this formula: concentration (µg/µl) = $Absorbance_{260}$ of diluted sample x 9.88 9.88 is a multiplication factor that accounts for the 1:200 dilution and the O.D. of the DNA in H_2O.

Reagent Preparation

50x TAE Buffer

Tris base	242 g
Glacial acetic acid	57.1 ml
0.5M EDTA (pH 8.0)	100 ml

Add distilled water (~600 ml) to a final volume of 1 liter. Dilute this solution to 1x before use.

Gel Preparation

Step	Action
1	Determine the volume of the gel and the volume of TAE running buffer needed for your electrophoresis apparatus. Prepare the gel casting tray of the apparatus.
2	Prepare a sufficient volume of 1x TAE Buffer for casting the gel and the running buffer by diluting the 50x TAE stock solution with dH_2O.
3	Add the appropriate volume of 1x TAE Buffer for the gel to a clean Erlenmeyer flask.
4	Weigh out a sufficient amount of the Electrophoresis Gel Powder for a 1.5% (w/v) gel and add it to the flask.
5	Swirl to suspend the Electrophoresis Gel Powder in the buffer and record the weight of the flask and solution.
6	Loosely cover the flask with plastic wrap and heat in a microwave oven on medium to medium-high until no particulates are visible in the molten gel. The time required will vary depending upon the microwave used and the gel volume. **NOTE:** Electrophoresis Gel Powder will require about one minute longer than agarose.
7	Weigh the flask and replace any water that was lost by evaporation during heating. Gently swirl the flask to mix. **CAUTION:** Handle microwaved liquids with care. The molten gel solution is very hot and may bubble over during mixing.
8	Once bubbles have stopped forming in the solution, wait an additional minute to pour the gel into the casting tray of your electrophoresis apparatus. Allow the gel to cool for 30 minutes.
9	For optimal results, place the gel at 4°C for 30 minutes before use.
10	Place the gel in the electrophoresis chamber. Flood the chamber and gel with the running buffer and remove the well-forming comb.

Troubleshooting

Problem: Low DNA recovery.

Cause	Remedy
Inadequate lysis of the sample.	Use a larger volume of Sample Buffer and Lysis Solution.

Problem: Phase inversion (step 9).

Cause	Remedy
Lysis Solution was not diluted sufficiently.	Make sure to add equal volumes of Lysis Solution to the sample in Sample Buffer.
Lysate was too viscous or there were too many cells in the sample.	Resuspend the sample in a larger volume of Sample Buffer and Lysis Solution.

Problem: Four phases observed during extraction (step 9).

Cause	Remedy
Initial cell number was greater than 10^8 cells/ml.	Repeat sample extraction with an appropriate increase in the reagent volumes to compensate for the greater cell number in the sample.

Problem: Cloudy aqueous phase.

Cause	Remedy
Interphase was removed with the aqueous phase.	If the interphase is accidentally removed, centrifuge the tube again and carefully remove the upper layer.
Carryover of the organic phase.	If the aqueous layer is extremely cloudy a chloroform extraction may be required. Add an equal volume (~400 µl) of chloroform to the aqueous layer in a fume hood. Vortex the sample for 10 seconds, centrifuge the tube for 10 minutes, and carefully transfer the upper aqueous layer to a fresh tube.

Problem: All samples show little or no apoptotic DNA fragmentation.

Cause	Remedy
Tissue culture samples may not be undergoing apoptosis.	Conditions for inducing apoptosis may need to be changed. These conditions will vary with cell type.
Apoptotic cells may have been washed away.	In adherent cell cultures, apoptotic cells may detach from the flask surface. Collect detached cells by centrifuging the decanted medium, then wash once with cold PBS and resuspend into the 100 µl Sample Buffer aliquot that is added to the remaining cells on the flask.
Samples may have been processed too early, before significant fragmentation of the chromosomal DNA.	Repeat the experiment, but take samples at later time points after induction of apoptosis.

Problem: All samples including the negative control show extensive DNA degradation (appears as a smear).

Cause	Remedy
Harsh treatment of the DNA during extraction.	If the DNA is isolated in a very vigorous manner, it may become sheared. The DNA should be isolated again from fresh samples, but under less aggressive isolation procedures.
The negative control is undergoing apoptosis.	Choose another negative control.

Problem: All samples including the negative control show an apoptosis ladder.

Cause	Remedy
The negative control is undergoing apoptosis.	Choose another negative control.

Problem: Unusual banding pattern.

Cause	Remedy
Ribosomal RNA present in the sample. Usually two bands are present.	Incubate DNA from Step 16 (DNA Isolation) at 37°C for 10 minutes prior to loading onto gel. The DNase-free Water contains RNase A.

Problem: Electrophoresis Gel Powder does not dissolve.

Cause	Remedy
TBE buffer was used in place of TAE buffer.	If TAE is not available, melt the Electrophoresis Gel Powder in dH_2O prior to adding TBE buffer.

RELATED PRODUCTS

Apoptosis Detection Products		
Annexin V Fluorescent Detection Kit	30-1236	100 tests
Anti-G3PDH Antibody	30-1255	100 µl
Anti-PARP Monoclonal Antibody	30-1253	50 µl
Anti-Phosphorylated Histone H2AX Polyclonal Antibody	30-1251	20 µl
Anti-Phosphorylated Histone H2AX Polyclonal Antibody	30-1252	100 µl
Apoptotic Cell DNA Laddering Kit	30-1231	20 tests
CV Caspase 3 & 7 Substrate Kit	30-1344	100 tests
FAM Caspase 3 & 7 Binding Kit	30-1304	100 tests
FAM Caspase 8 Binding Kit	30-1306	100 tests
FAM Caspase 9 Binding Kit	30-1308	100 tests
FAM Poly-Caspase Binding Kit	30-1302	100 tests
Mitochondrial Potential Assay Kit	30-1233	100 tests
PARP Activity Assay Kit	30-1235	50 tests
SR Caspase 3 & 7 Binding Kit	30-1324	100 tests
SR Poly Caspase Binding Kit	30-1322	100 tests
TdT Flow Cytometry Apoptosis Detection Kit	30-1207	60 tests
TdT In Situ Apoptosis Detection Blue Kit	30-1202	30 tests
TdT In Situ Apoptosis Detection DAB Kit	30-1201	30 tests
TdT In Situ Replenisher Kit	30-1205	30 tests
TdT Microplate Apoptosis Detection Kit	30-1206	96 tests
Stains and Reagents		
Erythrosin B Stain Solution	30-2404	40 ml
Trypan Blue Stain Solution	30-2402	40 ml
Dulbecco's Phosphate Buffered Saline (PBS)	30-2200	500 ml
Molecular Grade Water	60-2450	1 liter
Clone Sets		
Human Apoptosis Clone Set	MBA-89	
Mouse Apoptosis Clone Set	MBA-90	

ATCC also has apoptotic inducible cell lines, cell line model systems, and hybridomas, as well as a full line of products for cell culture. See our Web site at www.atcc.org for details.

Please refer to the Material Transfer Agreement and packing slip enclosed with the product for terms and conditions.

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