For life science research only.
Not for use in diagnostic procedures.

Apoptotic DNA-Ladder Kit

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Cat. No. 11 835 246 001 1 kit
20 tests

Store the kit at +15 to +25°C.

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1. General Information

1.1. Contents

Vial / Bottle	Cap	Label	Function / Description	Content
1	green	Apoptotic DNA-Ladder Kit, Binding/Lysis buffer	6 M guanidine-HCl, 10 mM urea, 10 mM Tris-HCl, 20% Triton X-100 (v/v), pH 4.4.	1 bottle, 20 ml
2	blue	Apoptotic DNA-Ladder Kit, Washing buffer	20 mM NaCl and 2 mM Tris-HCl, pH 7.5. *Final concentration after addition of 80 ml ethanol before use.*	1 bottle, 20 ml
3	colorless	Apoptotic DNA-Ladder Kit, Elution buffer	10 mM Tris, pH 8.5.	1 bottle, 40 ml
4	–	Apoptotic DNA-Ladder Kit, Filter tubes	Polypropylene tubes have two layers of glass fiber fleece and can hold up to 700 µl sample volume.	2 bags, 10 Filter tubes each
5	–	Apoptotic DNA-Ladder Kit, Collection tubes	2 ml polypropylene tubes.	2 bags, 10 Collection tubes each
6	red	Apoptotic DNA-Ladder Kit, Positive control	Lyophilized apoptotic U937 cells.	1 bottle

1.2. Storage and Stability

Storage Conditions (Product)

When stored at +15 to +25°C, the kit is stable through the expiration date printed on the label.

Vial / Bottle	Cap	Label	Storage
1	green	Binding/Lysis buffer	Store at +15 to +25°C.
2	blue	Washing buffer	
3	colorless	Elution buffer	
4	–	Filter tubes	
5	–	Collection tubes	
6	red	Positive control	

1.3. Additional Equipment and Reagent required

Standard laboratory equipment

- Standard benchtop centrifuge
- Vortex
- Pipettes (15 µl, 100 µl, 200 µl, 400 µl, and 500 µl)
- Sterile pipette tips
- Erlenmeyer flasks
- 1.5 ml tubes

For DNA isolation

- Ethanol, analysis grade
- Phosphate-buffered saline (PBS)*
- Isopropanol

For electrophoresis

- Agarose MP*
- Ethidium bromide or SYBR Green I Nucleic Acid Gel Stain
- Tris base*
- EDTA
- Boric acid
- NaOH
- Double-distilled water
- Sodium dodecyl sulfate (SDS)*
- Bromophenol blue
- Glycerol
- Microwave
- Electrophoresis chamber set
- DNA Molecular Weight Marker*

1.4. Application

The Apoptotic DNA-Ladder Kit is designed for the purification of nucleic acids from different sample materials, such as whole blood and cultured cells, to detect the typical DNA ladder which is the hallmark of apoptotic cells. DNA can be applied to the agarose gel directly after its elution from the column, resulting in a less time-consuming purification protocol compared to alternative methods which require extraction with organic solutions or DNA precipitation.

ℹ *Proteinase K treatment is not required, see section,* **Results**, *Figure 1.*

2. How to Use this Product

2.1. Before you Begin

Sample Materials

The Apoptotic DNA-Ladder Kit can be used with:
- Whole blood
- Cultured cells

Safety Information

- The Binding/Lysis buffer (Bottle 1) contains guanidine-HCl which is an irritant. Wear gloves and follow standard safety precautions when handling.
- Ethidium bromide is a mutagen and potential carcinogen. Wear gloves and follow standard safety precautions when handling ethidium bromide solutions.

For customers in the European Economic Area

Contains SVHC: octyl/nonylphenol ethoxylates. For use in research and under controlled conditions only – acc. to Art. 56.3 and 3.23 REACH Regulation.

Laboratory procedures

- Handle all samples as if potentially infectious, using safe laboratory procedures. As the sensitivity and titer of potential pathogens in the sample material varies, the operator must optimize pathogen inactivation by the Lysis / Binding Buffer or take appropriate measures, according to local safety regulations.
- Do not eat, drink or smoke in the laboratory work area.
- Do not pipette by mouth.
- Wear protective disposable gloves, laboratory coats and eye protection, when handling samples and kit reagents.
- Wash hands thoroughly after handling samples and reagents.

Waste handling

- Discard unused reagents and waste in accordance with country, federal, state, and local regulations.
- Safety Data Sheets (SDS) are available online on dialog.roche.com, or upon request from the local Roche office.

Working Solution

Solution	Preparation	For use in...
Washing buffer	▪ Add 80 ml analysis-grade ethanol to the Washing buffer (Bottle 2).	Washing steps
Positive control working solution	▪ Dissolve Positive control (Bottle 6) in 400 µl Binding/Lysis buffer (Bottle 1); mix immediately. ▪ Incubate 10 minutes at +15 to +25°C. ▪ After dilution, store at −15 to −25°C for 14 days.	Control reaction
0.5 M EDTA solution	▪ Dissolve 18.6 g EDTA in 80 ml double-distilled water and stir. ▪ Adjust pH 8.0 ± 0.1 with 1 M NaOH. *EDTA solubilizes at alkaline pH only.* ▪ After solubilization, dilute up to 100 ml with double-distilled water.	DNA gel electrophoresis
TBE buffer	▪ Dissolve 5.4 g Tris*, 2.8 g boric acid in 800 ml double-distilled water. ▪ Add 2 ml of 0.5 M EDTA solution. ▪ Stir until dissolved, final pH 8.0 ± 0.1. Dilute up to 1 liter with double-distilled water.	DNA gel electrophoresis
Ethidium bromide stock solution	▪ Dissolve 50 mg ethidium bromide in 5 ml double-distilled water. ***See section,* Safety Information *for handling precautions.*** *Alternatively, use SYBR Green I Nucleic Acid Gel Stain instead of ethidium bromide.*	DNA gel electrophoresis
Loading buffer (10x)	▪ Dissolve 0.1 g sodium dodecyl sulfate (SDS*), 25 mg Bromophenol blue in 7 ml double-distilled water. ▪ Add 3 ml glycerol.	DNA gel electrophoresis

2.2. Protocols

⚠ ***Before starting a purification reaction, warm the Elution buffer (Bottle 3) to +70°C; all other reagents should be at +15 to +25°C.***

Isolation of DNA from whole blood or cultured cells

1. Use 2 × 10^6 cells in a sample volume of 200 µl.
   ℹ *200 µl whole blood contains approximately 2 × 10^6 cells.*
   – Add up to 200 µl PBS to the sample material.
2. Add 200 µl Binding/Lysis buffer (Bottle 1) to a final volume of 400 µl; mix immediately.
3. Incubate for 10 minutes at +15 to +25°C.
   ℹ *If larger sample volumes will be used, increase all volumes accordingly. Up to 300 µl of whole blood can be used.*
4. After the 10 minute incubation to samples and Positive control, add 100 µl isopropanol and shake, for example, using a vortex.
5. Combine a Filter tube and a Collection tube and pipette the sample or the Positive control into the upper reservoir.
6. Centrifuge for 1 minute at 8,000 rpm in a standard benchtop centrifuge.
7. Discard the flow through and again combine the Filter tube and the used Collection tube.
   – Add 500 µl Washing buffer (Bottle 2) to the upper reservoir and centrifuge for 1 minute at 8,000 rpm.
8. Repeat Step 7.
   – Centrifuge for 10 seconds at maximal speed (13,000 rpm) to remove residual Washing buffer.
9. Discard the Collection tube and insert the Filter tube into a clean 1.5 ml cup or tube.
10. Elute DNA using 200 µl of prewarmed (+70°C) Elution buffer (Bottle 3).
    – A higher elution volume can be used to increase the elution efficiency, for example, 2 × 200 µl (excluding the Positive control).
    – Add Elution buffer to the Filter tube and centrifuge for 1 minute at 8,000 rpm.

ℹ *The DNA is stable and can be used directly or stored at −15 to −25°C for later analysis. Do not store >14 days.*

Preparation of the electrophoresis chamber

1. Seal the gel casting platform with adhesive tape if the ends are open.
2. To further prevent leakage, apply hot agarose using a Pasteur pipette to the joints and edges of the gel platform and allow to solidify.

Preparation of a 10 × 10 cm DNA 1% agarose gel

For additional information on preparing solutions, see section, **Working Solution**.

1. Add 1 g Agarose* into an Erlenmeyer flask and add 100 ml TBE Buffer.
   – Place the flask into a microwave oven and heat at maximum energy level. Observe the solution and shake when boiling occurs.
   ⚠ ***Handle carefully as superheating causes a delay in boiling.***

2. Microwave until agarose is completely dissolved; alternatively, autoclave flask to melt the agarose.
   – Cool solution to +60°C and add 5 µl Ethidium bromide stock solution.

3. Pour the solution into the prepared electrophoresis chamber and position the gel comb.

4. After the gel has solidified, remove the tape from the open ends of the gel platform, and carefully withdraw the gel comb without tearing the sample wells.

Sample preparation

For additional information on preparing solutions, see section, **Working Solution**.

1. Mix 1 to 3 µg of purified DNA and 15 µl of Positive control DNA with Loading buffer.
   Volume of DNA and Loading buffer prepared should not completely fill the wells.

2. Additionally, prepare a DNA Molecular Weight Marker* (2 µg) by mixing with Loading buffer.

Loading samples and running the agarose gel

For additional information on preparing solutions, see section, **Working Solution**.

1. Add sufficient TBE Buffer to cover the gel to a depth of approximately 1 mm.
   – Make sure no air pockets are trapped within the wells.
   – Plug in the electrophoresis box, attaching the leads so that the DNA will migrate into the gel towards the anode or positive lead.

2. Load samples carefully into the sample wells using a micropipette.

3. Turn on the power supply and set the voltage to 75 V (typically 5 V/cm) for 1.5 hours.

4. Turn off the power supply when the Bromophenol blue dye from the Loading buffer has migrated to the end of the platform.

5. Visualize the DNA by placing the gel onto a UV light source and photograph.
   ⚠ ***UV light is damaging to eyes and exposed skin. Always wear protective eyewear.***

6. If necessary, destain the gel by shaking in water for 30 minutes.

3. Results

Specimen	Sample Volume	Yield of DNA [µg]
Whole blood (human)	200 µl	3 – 6
K-562 cultured cells	2 × 10^6 cells	20
U937 cultured cells	2 × 10^6 cells	12

Apoptotic DNA ladder

A clear DNA ladder is visible when DNA is isolated out of 2 × 10^6 U937 cells treated with 4 µg/ml camptothecin (CAM) for 3 hours, usually resulting in about 30% apoptotic cells (Fig. 1). Load agarose gel with 3 µg purified DNA in loading buffer.

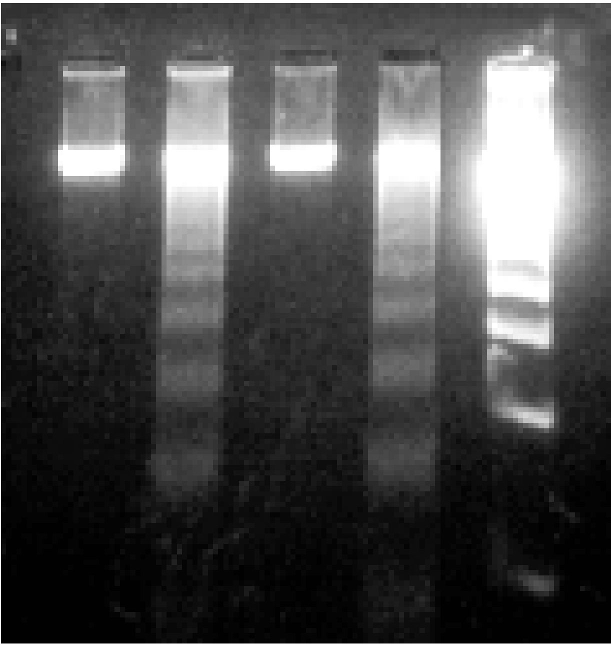

Fig. 1: Example of DNA ladder.
Lane 1: U937 cells without CAM.
Lane 2: U937 cells with CAM.
Lane 3: U937 cells without CAM (Proteinase K* treated).
Lane 4: U937 cells with CAM (Proteinase K* treated).
Lane M: Marker
Result: These is no difference between treatment with Proteinase K and untreated samples.

4. Troubleshooting

Observation	Possible cause	Recommendation
No DNA ladder; loading band visible.	No or not enough apoptosis induced.	Use higher concentrations of apoptosis-inducing agents.
		Increase incubation interval.
No DNA ladder; loading band not visible.	Not enough DNA isolated.	Use more cells (use same volume but load filter column twice).
		Centrifugation at Step 5 (13,000 rpm, 10 seconds) was too short or not excessive enough to remove the ethanol.
DNA smear visible; no laddering.	Nonspecific cleavage due to late apoptosis.	Isolate DNA at an earlier stage (perform kinetics).
DNA smear visible; weak laddering of Positive control.	Overlay of RNA.	Incubate purified DNA with 2 µg/ml RNase, DNase free* for 20 minutes at +15 to +25°C.
DNA ladder visible in non-apoptotic cell populations.	Mycoplasma contamination; mycoplasma sourced nucleases generate a DNA cleavage similar to an apoptotic DNA ladder.	Perform mycoplasma elimination.
Positive control visible; bands are weak.	Some of the Positve control remained in Bottle 6.	Resuspend the cell pellet thoroughly by pipetting.
		Rinse Bottle 6 with an additional 200 µl Binding/Lysis buffer and add it to the Filter tube.

5. Additional Information on this Product

5.1. Test Principle

How this product works

1. Lysis of blood or cultured cells in Binding/Lysis buffer.

2. Lysate applied to a filter tube with glass fiber fleece and passaged through the fleece by centrifugation.

3. Nucleic acids bind specifically to the surface of the glass fibers in the presence of chaotropic salts.

4. Residual impurities are removed by a wash step and DNA is eluted in Elution buffer.

6. Supplementary Information

6.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

6.2. Changes to previous version

Layout changes.
Editorial changes.
New information added related to the REACH Annex XIV.

6.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
RNase, DNase-free, High Concentration	1 mg, 10 mg/ml	11 579 681 001
Sodium Dodecyl Sulfate (SDS)	1 kg	11 667 289 001
Buffers in a Box, Premixed PBS Buffer, 10x	4 l	11 666 789 001
DNA Molecular Weight Marker IX	50 µg, 1 A_{260}, 200 µl, 50 gel lanes	11 449 460 001
Agarose MP	100 g	11 388 983 001
	500 g	11 388 991 001
Tris base	1 kg, *Not available in US*	10 708 976 001
	1 kg	03 118 142 001
	5 kg	11 814 273 001
Proteinase K, recombinant, PCR Grade	1.25 ml, > 50 U/ml	03 115 887 001
	5 ml, > 50 U/ml	03 115 828 001
	25 ml, > 50 U/ml	03 115 844 001

6.4. Trademarks

SYBR is a trademark of Thermo Fisher Scientific Inc..
All other product names and trademarks are the property of their respective owners.

6.5. License Disclaimer

For patent license limitations for individual products please refer to:
List of biochemical reagent products.

6.6. Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

6.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

6.8. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications,
please visit our **Online Technical Support Site**.

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed.

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