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TACS® Apoptotic DNA Laddering Kit

Protocol Covers the Following:

Cat# 4850-20-ET	Ethidium Bromide	20 reactions
Cat# 4859-20-K	Tissue Supplement	20 reactions

TACS® Apoptotic DNA Laddering Kit

Cat# 4850-20-ET
Cat# 4859-20-K

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I. Background

Trevigen's **TACS® Apoptotic DNA laddering kit** is used to assay cells and tissues for apoptosis by detecting double-strand breaks in genomic DNA and determining the level of DNA degradation. DNA fragmentation is considered to be irreversible, and a classical indicator of apoptosis. During apoptosis, DNA is cleaved between the nucleosomes into 180-200 bp fragments. These DNA fragments are analyzed by horizontal gel electrophoresis followed by ethidium bromide staining.

When analyzing tissue samples, it is necessary to disrupt the tissue and create a cell suspension. The Tissue Supplement Kit (Cat# 4859-20-K), available separately, provides the reagents to allow DNA laddering analysis in tissues.

II. Precautions and Limitations

1. For Research Use Only. Not for use in diagnostic procedures.
2. Some components such as Lysis and Extraction Solutions are harmful if ingested or inhaled and maybe 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 MSDS for further information on kit components.
3. The physical, chemical, and toxicological properties of the products contained in the TACS® Apoptotic DNA Laddering Kit may not yet have been fully investigated. Therefore, Trevigen recommends the use of gloves, lab coats, and eye protection while using any of these chemical reagents. Trevigen assumes no liability for damage resulting from handling or contact with these products.

III. Materials Supplied

Component	Catalog Number	Volume	Storage Temperature	EtBr[1]	Tissue Suppl.
Lysis Solution 1	4850-20-01	2 x 1 ml	Room temp.	•	
Extraction Solution 2	4850-20-02	20 ml	Room temp.	•	
Extraction Buffer 3	4850-20-03	8 ml	Room temp.	•	
Sodium Acetate 4	4850-20-04	1 ml	Room temp.	•	
DNase-free Water 5	4850-20-05¥	2 ml	Room temp.	•	
Sample Buffer 6	4850-20-06*	2 ml	Room temp.	•	•
5X Gel Loading Buffer	4850-20-10	250 µl	Room temp.	•	
TreviGel™ 500 Powder	9804-06-P	6 gm	Room temp.	•	
10X Tissue Buffer	4859-20-01*	500 µl	-20 °C		•

1: EtBr = 4850-20-ET Ethidium Bromide
Tissue Suppl. = 4859-20-K Tissue Supplement
¥Contains RNase A.
*These reagents are required for extracting DNA from tissue.

IV. Materials Required But Not Supplied

Equipment:

1. 1-20 µl, 20-200 µl, and 200-1000 µl pipettors, and tips
2. microcentrifuge
3. 65 °C water bath
4. electrophoresis apparatus with power supply
5. vortex
6. -20 °C freezer for storage of components
7. UV spectrophotometer

Materials:

1. 1.5 ml microcentrifuge tubes
2. gloves (*i.e.* latex)
3. 1, 5, and 10 ml pipettes

Reagents:

1. 2-propanol (isopropanol)
2. 70% Ethanol
3. 10X PBS (Cat# 4870-500-6)
4. 50X TAE (Cat# 9860-500-2)
5. ethidium bromide
6. Deionized water

V. Reagent Preparation

Note: Wear gloves, lab coat, and eye protection when handling any chemical reagents.

1. 1X PBS, pH 7.4

Dilute 10X PBS with deionized water to prepare 1X PBS. Store at room temperature. (10X PBS is available from Trevigen, Cat# 4870-500-6)

2. 1X TAE

Dilute 50X TAE with deionized water to prepare 1X TAE pH = 8.2. Store at room temperature. (50X TAE is available from Trevigen, Cat# 9860-500-2). The standard working solution (1X TAE) is 40 mM Tris-acetate and 1 mM EDTA.

VI. Assay Protocols

A. DNA Isolation from Tissues:

Purchase Tissue Supplement Reagents (Cat# 4859-20-K) separately.
Note: Before opening tubes, briefly centrifuge to bring contents to the bottom.

It is recommended that you execute the entire protocol without stopping. If necessary, samples may be frozen at -20 or -80 °C after steps 3 or 9 of the DNA isolation procedure. Cell suspensions prepared from tissue using the Tissue Supplement Kit (4859-20-K) may be stored by freezing after step 3 of that procedure.

If processing cells from culture, proceed to DNA isolation from cultured cells. For best results, process the tissue sample soon after removal from the animal.

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1. Mince the tissue into smaller pieces and freeze in liquid nitrogen.

2. Grind the frozen tissue samples into powder using either a pre-chilled hammer or mortar and pestle to avoid thawing of the sample. Add additional liquid nitrogen if required. Process 0.2-1 gram of the powder at one time. Please consider the amount of material being processed as only 20 x 100 µl samples can be processed in the DNA Laddering Kit.

3. Suspend the tissue into Sample Buffer in the following ratio:

   0.1 g of powdered tissue sample
   200 µl of Sample Buffer (Cat# 4850-20-06)
   20 µl of 10X Tissue Buffer (Cat# 4859-20-01)

   Allow the tissue to thaw and incubate at 50 °C for 12 to 18 hours. Gentle shaking is recommended.

4. Transfer 100 µl of the cell suspension from step 3 to a new microcentrifuge tube. Add 100 µl Lysis Solution 1 (Cat# 4850-20-01) provided with the DNA Laddering Kit and mix thoroughly by inverting tube several times.
   Note: The remaining cell suspension may be frozen at -80 °C for future use.

5. Continue with step 4 of part VI B: *DNA Isolation from Cultured Cells*

B. DNA Isolation from Cultured Cells:

Note: For best results, process samples immediately after harvesting.

1. ***Suspension Cells:*** Harvest cells (10^5 to 10^7) by centrifugation and resuspend the pellet in 100 µl of Sample Buffer (10^6 to 10^8 cells/ml). Proceed to step 2.

   Adherent Cells: Lyse cells directly in culture dish by adding 100 µl of Sample Buffer and 100 µl of Lysis Solution 1. Gently scrape cells and pipette until completely in solution. Proceed to step 4.

2. Incubate this cell suspension at room temperature for 5 to 15 minutes.

3. Lyse the sample and stabilize the DNA by adding 100 µl of Lysis Solution 1. Mix thoroughly by inverting tube several times.

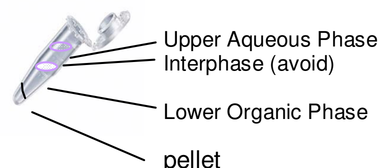
4. Make sure that the sample is now in a 1.5-2 ml microcentrifuge tube. Shake Extraction Solution 2 and then add 700 µl to the sample.

5. Add 400 µl of Extraction Buffer 3 to the sample. Vortex for 10 seconds.

6. Centrifuge at 12,000 x g for 5 minutes.

7. Transfer the upper layer (aqueous) to a new microcentrifuge tube. Avoid removing the darker, lower organic and interphase layer. If the recovered aqueous layer is cloudy, repeat steps 6 and 7. Discard the organic layer by protocols established in your laboratory.

   Note: If the aqueous layer is very cloudy, a chloroform extraction may be required. This can be performed by adding 400 µl of chloroform to the aqueous layer in a fume hood. Vortex the sample for 10 seconds, and repeat steps 6 and 7.



When removing the upper phase:
Carefully remove the upper aqueous phase without disturbing the interphase.

If the interphase is accidentally removed, centrifuge the tube again and carefully remove the upper layer.

8. Add 40 µl (*i.e.* 0.1 volume) of Sodium Acetate 4 to the aqueous sample that was transferred. Mix by inverting.

9. Add 440 µl (*i.e.* equal volume) of 2-propanol and mix by inverting.
   Note: Optional stopping point by placing samples at -20 °C overnight.

10. Centrifuge at 12,000 x g for 10 minutes at room temperature.

11. 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.

12. Centrifuge at 12,000 x g for 5 minutes at room temperature.

13. Carefully remove the supernatant without disturbing the pellet. Allow the pellet to dry by inverting the tube on a laboratory tissue and allowing the liquid to evaporate, or with the aid of a vacuum centrifuge apparatus.

14. Resuspend the pellet in 100 µl of DNase-free Water 5.

15. Quantitate the DNA spectrophotometrically. Take 5 µl of the DNA sample and add to 1 ml of water. Determine the optical density at 260 nm against a water blank. The DNA concentration of the undiluted sample can be determined as:

    concentration (µg/µl) = $Absorbance_{260}$ of diluted sample x 9.88*

    * The 9.88 is a multiplication factor that accounts for the 1:200 dilution and the O.D. of the DNA in H_2O.

    Alternatively, the DNA concentration can be estimated from an ethidium bromide stained gel by comparing with a sample of DNA of known concentration.

C. Assay Procedure:

1. To 1-2 µg of isolated DNA, add sufficient water (DNase-free) to make volume add up to 10 µl. Add 2 µl of 5X Gel Loading Buffer (provided with kit). Load onto

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a 1.5% TreviGel™ 500 gel in 1X TAE buffer. For gel preparation, refer to the end of this protocol, "Casting TreviGel 500," on page 10. Separate the DNA fragments by electrophoresis (typically at 100 V until the bromophenol blue dye front reaches the lower third of the gel).

2. Remove the gel from the apparatus and immerse gel in 0.5 µg/ml ethidium bromide prepared in dH_2O or 1X TAE buffer for 15 minutes and destain in dH_2O or 1X TAE Buffer until the background staining has disappeared.

3. View under UV light with eye protection.
   Caution: Wear gloves when handling solutions or gels containing ethidium bromide. Visualize DNA by illumination on a UV light source. Wear protective goggles and clothing during exposure to UV light.

VII. Interpretation

In the majority of cell types, a typical ladder pattern of bands in multiples of approximately 200 base pairs is a hallmark of apoptosis. Although the method is not quantitative, qualitative comparisons can be made with control cells and between samples prepared from equivalent cell numbers. Faint DNA laddering patterns may be evident in control samples due to the low level of apoptosis that normally takes place in many cells in culture. The appearance of 2 distinct bands when using the Ethidium Bromide kit may indicate the presence of RNA contamination. Refer to the Troubleshooting Guide on page 11 for information on removing RNA.

VIII. Casting TreviGel™ 500

TreviGel 500 powder can be used to prepare horizontal gels for standard laboratory purposes. The following method for TAE gel casting gives consistent, high resolution results. For high efficiency transfer out of gels, thinner gels are recommended (Transfer efficiency α 1/distance), therefore, it is preferable to use one-half the volume typically used in your gel apparatus.

1. Weigh out enough TreviGel 500 for the volume of gel required. For example, 100 ml of a 1.5% gel is made by weighing 1.5 g of TreviGel 500 powder.

2. Prepare enough 1X TAE buffer for both casting and running of the gel. Add the 1X TAE buffer to a clean Erlenmeyer flask (100 ml in this example). Transfer the TreviGel™ powder to the Erlenmeyer flask and swirl to make certain that the gel is in suspension. Place the flask on a top loading balance and tare or record the weight.

3. Microwave on medium to medium-high setting until no particulates are visible in the molten gel. The time will vary since microwave units are not consistent in power. In general, TreviGel™ 500 requires heating for about one minute longer than agarose. Gently swirl flask to mix. HANDLE WITH CARE - the molten TreviGel™ 500 is very hot. Replace water that evaporated during microwaving by returning to top loading balance and adding distilled water until the tared weight (or recorded weight) is reached. Gently swirl to mix.

4. Once bubbles have stopped forming, wait an additional minute to pour the gel into a casting tray. Allow the gel to cool for 30 minutes.

5. For optimal results, place gel at 4 °C for 30 minutes before electrophoresing samples.

6. To each labeled DNA sample (10 µl), add 2 µl of 5X gel loading dye. Load samples into wells and electrophorese at 50 to 100 V for about 2 hours. Conditions may vary with electrophoresis setup used.

IX. Troubleshooting Guide

PROBLEM	POSSIBLE CAUSE	RECOMMENDATIONS
Precipitate in Lysis Solution	Solution was chilled	Warm to 37°C and mix by inversion until all precipitates are dissolved
DNA recovery	Inadequate lysis of sample	Mix equal volumes of Lysis Solution 1 with sample in Sample Buffer
Phase inversion	Lysis solution 1 not diluted sufficiently	Make sure to add equal volumes of Lysis Solution 1 with the sample in Sample Buffer.
	Lysate too viscous	Resuspend the sample in a larger volume of Sample Buffer and Lysis solution 1.
All samples show little or no apoptotic DNA fragmentation	Tissue culture samples may not be undergoing apoptosis.	Conditions for inducing apoptosis may need to be changed. These conditions will vary with cell type.
Unusual banding pattern.	Ribosomal RNA present in sample. Usually 2 bands are present	Incubate DNA from Step 14 on p. 6 at 37 °C for 10 minutes prior to loading onto gel.
All samples including the negative control show extensive DNA degradation (Appears as a smear).	Harsh treatment of DNA	If the DNA is isolated in a very vigorous manner, the DNA may become sheared. The DNA should be isolated again from fresh samples, but under less aggressive isolations procedures.
	Negative control is inappropriate	The negative control may be undergoing apoptosis and may therefore be inappropriate as a control. The choice of an alternative negative control may be warranted.

X. References

1. Rösl, F. 1992. A simple method for detection of apoptosis in human cells. *Nucleic Acid Research* **201**:5243.
2. Smith, M.L. and A.J. Fornace. 1996. Mammalian DNA damage-inducible genes associate with growth arrest and apoptosis. *Mutat Res* **340**:109-124.
3. Pinsky, D.J. et al. 1999. Nitric oxide triggers programmed cell death (apoptosis) of adult rat ventricular myocytes in culture. *Am J Physiol Heart Circ Physiol* 277:1189-1199.

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XI. Related products available from Trevigen.

Catalog #	Description	Size
4817-60-K	FlowTACS™ Kit	60 samples
4822-96-K	HT TiterTACS™ Assay Kit	96 tests
4830-01-K	TACS® Annexin V FITC Kit	100 samples
4835-01-K	TACS® Annexin V Biotin Kit	100 samples
6300-100-K	DePsipher™ Mitochondrial Potential Assay Kit	100 tests
6305-100-K	MitoShift™ Mitochondrial Potential Assay Kit	100 tests
4815-30-K	TumorTACS™ *In Situ* Apoptosis Detection Kit	30 samples
4823-30-K	NeuroTACS™ *In Situ* Apoptosis Detection Kit	30 samples
4829-30-K	DermaTACS™ *In Situ* Apoptosis Detection Kit	30 samples
4826-30-K	VasoTACS™ *In Situ* Apoptosis Detection Kit	30 samples
4827-30-K	CardioTACS™ *In Situ* Apoptosis Detection Kit	30 samples
4828-30-DK	TACS•XL® DAB *In Situ* Apoptosis Detection Kit	30 samples
4828-30-BK	TACS•XL® Blue Label *In Situ* Apoptosis Detection Kit	30 samples
4810-30-K	TACS® 2 TdT DAB *In Situ* Apoptosis Detection Kit	30 samples
4811-30-K	TACS® 2 TdT Blue Label *In Situ* Apoptosis Detection Kit	30 samples
4812-30-K	TACS® 2 TdT Fluorescein Apoptosis Detection Kit	30 samples
4892-010-K	Cultrex® Calcein-AM Cell Viability Kit	1000 tests
4684-096-K	HT Colorimetric PARP Apoptosis Assay	96 samples
4685-096-K	HT Chemiluminescent PARP Apoptosis Assay	96 samples

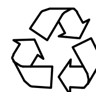
Antibodies

Catalog #	Description	Size
4411-PC-100	Anti-Phosphorylated Histone-γ-H2AX polyclonal	100 µl
2291-MC-100	Anti-human-Bcl-2 mAb (clone YTH-8C8)	100 µg
2281-MC-100	Anti-human-Bax mAb (clone YTH-6A7)	100 µg
6361-PC-100	Anti-human/mouse-PBR polyclonal	100 µl
6370-MC-100	Anti-human/murine-Cytochrome C	100 µg
6380-MC-100	Anti-human/murine-Holocytochrome C	100 µg
4335-MC-100	Anti-PAR polymer mAb (10HA)	100 µl
4336-BPC-100	Anti- PAR polymer polyclonal	100 µl
4338-MC-50	Anti-human/murine-PARP mAb (clone C2-10)	50 µg

Accessories

Catalog #	Description	Size
4800-30-40	Tissue Control Slides	2 ea.
4800-30-20	Cell Culture Control Slides	2 ea.
4864-100	Treated Glass Microscope Slides w/3 sample Hydrophobic Barrier	100 ea.
4867-100	Hydrophobic Coverslips	100 ea.
4862-10	Coverslips 24 x 60 mm, No. 1.5	10 oz.
4865-25	Mounting Medium	25 ml
4800-30-14	Strep-Fluorescein	30 µl
4830-250-03	Propidium Iodide	2.5 ml
4870-500-6	10X PBS	6 x 500 ml
4869-500-6	Apoptosis Grade™ H_2O	6 x 500 ml
4820-30-13	Blue Counterstain	50 ml
4878-05-02	Cytonin™ IHC	2 x 5 ml

The product accompanying this document is intended for research use only and is not intended for diagnostic purposes or for use in humans.



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Trevigen, Inc.
8405 Helgerman Ct. Gaithersburg, MD 20877
Tel: 1-800-873-8443 • 301-216-2800
Fax: 301-560-4973
e-mail: info@trevigen.com
www.trevigen.com