DNA Laddering Kit

Item No. 660990

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Technical Support 888.526.5351
1180 E. Ellsworth Rd · Ann Arbor, MI · USA

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GENERAL INFORMATION

Materials Supplied

Item Number	Item	Quantity/Size	Storage
660991	Lysis Buffer	4 vials/1.2 ml	-20°C
660992	10% SDS	1 vial/0.48 ml	-20°C
660993	Enzyme A	1 vial/0.48 ml	-20°C
660994	Enzyme B	1 vial/0.48 ml	-20°C
660995	Precipitating Reagent	3 vials/1.04 ml	-20°C
660996	Gel-Loading Buffer (6X)	1 vials/0.48 ml	-20°C

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Fax: 734-971-3641

E-Mail: techserv@caymanchem.com

Hours: M-F 8:00 AM to 5:30 PM EST

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored at -20°C and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. 1.5 ml microcentrifuge tubes
2. 37°C incubator
3. 56°C incubator
4. Vortex mixer
5. Variable speed microcentrifuge
6. TE buffer
7. PBS buffer
8. 100% ethanol
9. 80% ethanol
10. Agarose
11. TBE buffer (89 mM Tris, 89 mM borate, 2 mM EDTA, pH 8.0)
12. Ethidium bromide (10 mg/ml)
13. Adjustable pipettor

For visualization of DNA fragmentation other instruments are required such as an ultraviolet transilluminator and gel documentation system.

INTRODUCTION

Background

Apoptosis, or programmed cell death, is a physiological mechanism of cellular demise required for the normal development and function of an organism. Dysregulation of the apoptotic pathway can contribute to the pathology of many diseases ranging from cancer to AIDS.[1] Fragmentation of chromatin DNA at the nucleosomal level (~185 bp) is one of the hallmarks of apoptosis.[2] This DNA fragmentation is regulated by at least one apoptosis-specific nuclease, CAD/CPAN/DFF40, and can be detected as laddering using gel electrophoresis.[3-5]

About This Assay

Cayman's DNA Laddering Kit allows the selective extraction of fragmented DNA with minimum contamination by intact chromatin. This kit is designed for detection of DNA laddering by gel electrophoresis.

Our DNA Laddering Kit has been validated for use on cultured cells. We recommend the prompt extraction of DNA following sample collection. Freeze-thawing has been demonstrated to dramatically decrease the yield of fragmented DNA obtained from cell samples. The quantity and quality of DNA obtained with Cayman's DNA Laddering Kit depends on a number of factors including cell type, method of induction of apoptosis, and time of sampling. Table 1, on page 7, gives examples of DNA obtained using this kit.

Cell Type	Method of Induction of Apoptosis	Number of Cells Assayed	Quantity of DNA Obtained	A_{260}/A_{280}
P3U1	Actinomycin D (10 µM) for 20 hours	1 x 10^6	>10 µg	>1.8
HL60	Staurosporin (1 µM) for 5 hours	1 x 10^6	>12 µg	>1.8
U937	TNF-α (2 ng/ml) and cyclohexamide (0.5 µg/ml) for 3 hours	1 x 10^6	>7 µg	>1.8

Table 1. Quantity and quality of DNA obtained from cultured cells using Cayman's DNA Laddering Kit

ASSAY PROTOCOL

Performing the Assay

NOTE: This protocol is appropriate for detection of apoptosis in 1 x 10^5 to 1 x 10^6 cells, approximately the number of cells on a 35 mm tissue culture dish.

1. **For adherent cultures:** Remove the culture medium. Wash the cells once with PBS, then scrape the cells into 250-500 µl PBS and transfer to 1.5 ml centrifuge tubes. Centrifuge at 1,600 x g for five minutes at room temperature.

   For suspension cultures: Collect cells by centrifugation at 1,600 x g for five minutes at room temperature. Decant supernatants. Resuspend cells in 1 ml PBS and transfer to 1.5 ml centrifuge tubes. Centrifuge at 1,600 for five minutes at room temperature.
2. Discard the supernatants and loosen the pellets by tapping the bottom of the tubes. Add 100 µl Lysis Buffer (Item No. 660991) and vortex for 10 seconds.
3. Centrifuge at 1,100 x g for five minutes at room temperature. Transfer the supernatants to clean microcentrifuge tubes.
4. Repeat DNA extraction from the cell pellets as described in steps 2 and 3, combining the supernatants from the two centrifugation steps. The remaining pellets may be discarded.
5. Add 20 µl 10% SDS (Item No. 660992) to each sample. Vortex briefly and add 20 µl Enzyme A (Item No. 660993). Vortex and incubate the samples at 56°C for one hour.
6. Add 20 µl Enzyme B (Item No. 660994) to each sample. Vortex to mix and incubate the samples at 37°C for one hour.
7. Add 130 µl Precipitating Reagent (Item No. 660995) and 950 µl ice-cold ethanol to each tube. Vortex to mix, then centrifuge at 12,000 x g for 15 minutes at 4°C.
8. Discard supernatant and wash pellet 1 x with ice-cold 80% ethanol. Repeat centrifugation as in step 7.
9. Briefly dry the pellets (5-10 minutes on the bench). Resuspend each sample in 50-100 µl TE by vigorous vortexing.
10. Mix 5 volumes sample with one volume Gel-Loading Buffer (6X) (Item No. 660996). 5-15 µl of sample prepared with this kit is usually sufficient to detect laddering by gel electrophoresis. *NOTE: The Gel-Loading Buffer (6X) included in Cayman's DNA Laddering Kit contains orange G rather than bromophenol blue as a tracking dye. The orange G migrates more quickly than the nucleosomal fragments and will not interfere with visualization of the apoptotic ladder as may happen if bromophenol blue is used as the tracking dye.*)

Troubleshooting

Problem	Possible Causes	Recommended Solutions
A DNA smear rather than a ladder is observed on electrophoresis	A. Apoptosis has progressed too far prior to sampling B. The sample is contaminated with DNases	A. Sample at an earlier time point B. Wear gloves and take other precautions to avoid contamination with DNases
No DNA band is observed on electrophoresis	A. Apoptosis did not occur B. DNA quantity was lower than limits of detection	A. Confirm apoptosis using another method of detection B. Increase the quantity of cells treated C. Concentrate the DNA solution by ethanol precipitation
SDS remains precipitated when thawed to room temperature	Not warmed up properly	Thaw precipitant by warming the solution to 40°C before use

References

1. Reed, J.C. *J. Cell Biol.* **124**, 1-6 (1994).
2. Wyllie, A. *Nature* **391**, 20-21 (1998).
3. Enari, M., Sakahira, H., Yokoyama, H., *et al. Nature* **391**, 43-50 (1998).
4. Halenbeck, R., MacDonald, H., Roulston, A., *et al. Curr. Biol.* **8**, 537-540 (1998).
5. Liu, X., Li, P., Widlak, P., *et al. Proc. Natl. Acad. Sci. USA* **95**, 8461-8466 (1998).

NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.