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ab139479 Chromatin Condensation Assay Kit

For the measurement of chromatin condensation in live cells.

View kit datasheet: www.abcam.com/ab139479
(use www.abcam.cn/ab139479 for China, or www.abcam.co.jp/ab139479 for Japan)

This product is for research use only and is not intended for diagnostic use.

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1. Overview

Chromatin Condensation Assay Kit (ab139479) provides a convenient approach for analysis of late stage apoptosis by flow cytometry and microscopy.

The basis of the assay is that the compacted chromatin of apoptotic cells binds higher amounts of nuclear dye compared to the healthy cells. The kit is suitable for differentiating between healthy and apoptotic cells with condensed nuclei.

Treat cells with experimental test agent of your choice.

Detach cells from plate if necessary (adherent cells).

Wash with 1X Assay buffer.

Incubate cells with DNA staining solution (30 min, 4°C in the dark).

Analyse by flow cytometry, fluorescence/confocal microscopy or microplate assay.

2. Materials Supplied and Storage

Store kit at -20°C in the dark immediately on receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.

Aliquot components in working volumes before storing at the recommended temperature.

Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
Green Detection Reagent	100 µL	-20 °C	-20 °C
Apoptosis Inducer (Staurosporine)	50 nmol	-20 °C	-20 °C
10X Assay Buffer	30 ml	-20 °C	-20 °C

3. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Flow cytometer equipped with 488 nm laser source.
- Microscope equipped with standard FITC filters.
- Tubes appropriate for holding cells for the flow cytometer.
- Calibrated, adjustable precision pipettes, preferably with disposable plastic tips.
- Adjustable speed centrifuge with swinging buckets (for suspension cultures).
- Deionized water.
- Anhydrous DMSO.
- Total growth medium suitable for cell type.
- 10% Triton X-100 in water (for Sub G0 analysis of permeabilized cells, optional).

4. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:
www.abcam.com/assaykitguidelines

For typical data produced using the assay, please see the assay kit datasheet on our website.

5. Reagent Preparation

- Allow all reagents to thaw at room temperature before starting with the procedures.
- Upon thawing, gently hand-mix or vortex the reagents prior to use to ensure a homogenous solution.
- Briefly centrifuge the vials at the time of first use, as well as for all subsequent uses, to gather the contents at the bottom of the tube.

5.1 Apoptosis Inducer

The Apoptosis Inducer (Staurosporine) is supplied as a lyophilized powder (50 nmoles) and should be reconstituted in 50 µL DMSO for a 1 mM stock solution. It is recommended that with the apoptosis inducer of choice, induction occurs at the effective concentration (EC_{50}) and that the final percent DMSO in the assay not exceed 0.2%.

5.2 10X Assay Buffer

Allow the 10X Assay Buffer to warm to room temperature. Make sure that the reagent is free of any crystallization before dilution. Prepare enough 1X Assay Buffer for the number of samples to be assayed by diluting each milliliter (mL) of the 10X Assay Buffer with 9 mL of deionized water.

5.3 Green Detection Reagent

The concentration of Green Detection Reagent for optimal staining will vary depending upon the application. Suggestions are provided to use as guidelines, though some modifications may be required depending upon the particular cell type employed and other factors such as the permeability of the dye to the cells or tissues. To reduce potential artifacts from overloading the cells, the concentration of the dye should be kept as low as possible. Prepare a sufficient amount of the staining solution for the number of samples to be assayed.

6. Sample Preparation

General sample information:

We recommend that you use fresh samples for the most reproducible assay.

6.1 Cell preparations

Positive control cells should be pretreated with Apoptosis inducer for 1-4 hours. Response to Staurosporine is time and concentration dependent and may also vary significantly depending upon cell type and cell line. Negative control cells should be treated with a vehicle (DMSO, media or other solvent used to reconstitute or dilute an inducer or inhibitor) for an equal length of time under similar conditions.

7. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature just prior to use and gently agitate.
- Assay all standards, controls and samples in duplicate.

7.1 Staining live cells for flow cytometry:

1. Cells should be maintained via standard tissue culture practice. Grow cells overnight to log phase in a humidified incubator at 37°C, 5% CO_2.
2. Treat cells with compound of interest and negative control cells with vehicle.
3. Prepare positive control cells by incubating with Apoptosis inducer (0.5-2 µM, see Reagent Preparation section) for 1-4 hours under normal tissue culture conditions.
4. At the end of treatment, trypsinize (adherent cells), or collect cells (suspension cells). Samples may contain 1 x 10^5 to 1 x 10^6 cells.
5. Centrifuge at 400 x *g* for 5 minutes to pellet the cells. Resuspend in media, 1X Assay Buffer, or other buffer of choice and centrifuge as before.
6. Immediately prior to staining the live cell samples, prepare fresh DNA Staining Solution as follows:

ΔNote: A concentration of 1-5 µM Green Detection Reagent is recommended for Chromatin Condensation analysis. The procedure described below is for preparation of 5 µM dye solution.

For each sample to be stained, dilute 1 µL Green Detection Reagent to a final volume of 1 mL with cold media or buffer of choice. Mix well and keep on ice.

7. Resuspend each live cell sample in 0.5 mL of freshly prepared cold DNA Staining Solution (from step 6). Incubate for 30 minutes at 4°C in the dark. It is important to achieve a monodisperse cell suspension at this step by gently pipetting up and down repeatedly.

8. Analyze the samples in the FL1 channel of a flow cytometer.

7.2 Staining permeabilized cells for sub-G_0 analysis by flow cytometry:

Sub-G_0 analysis of apoptotic cells, a measure of DNA fragmentation, may be performed using the Chromatin Condensation Assay Kit simply by permeabilizing the cells. The Green Detection Reagent intercalates with the chromosomal DNA and a population of lower-fluorescence fragmented DNA can be analyzed as follows.

1. Grow cells overnight to log phase in a humidified incubator at 37°C, 5% CO_2.
2. Treat cells with or without experimental compounds.
3. Prepare positive control cells by incubating with pre-diluted Staurosporine (0.5 - 2 µM, see Reagent Preparation section) for 1-4 hours under normal tissue culture conditions.
4. At the end of treatment, trypsinize (adherent cells), or collect cells (suspension cells). Samples may contain $1x10^5$ to $1x10^6$ cells.
5. Centrifuge at 400 x *g* for 5 minutes to pellet the cells. Re-suspend in media, 1X Assay Buffer, or other buffer of choice and centrifuge as before.
6. Immediately prior to staining the cell samples, prepare fresh DNA Staining/Permeabilizing solution as follows:

ΔNote: A concentration of 1-5 µM Green Detection Reagent is recommended for permeabilized cells. The procedure described below is for preparation of 5 µM dye solution.

For each sample to be stained, dilute 1 µL Green Detection Reagent and 10 µL of 10% Triton X-100 to a final volume of 1 mL with media or buffer of choice.

7. Re-suspend each fixed cell sample to be fixed in 0.5 mL freshly prepared DNA Staining/Permeabilizing Solution (from step 6) to a final concentration of $1x10^5$ to $1x10^6$ cells/mL.
8. Incubate for 30 minutes at 37°C in the dark.
9. Analyze the samples in the FL1 channel of a flow cytometer with a 488 nm excitation laser.

7.3 Wide field fluorescence/confocal microscopy:

1. Grow cells directly onto glass slides.
2. Treat cells with compound of interest and negative control cells with vehicle.
3. Carefully wash cells twice with ice-cold 1X Assay Buffer in a volume sufficient for covering the cell monolayer.
4. Carefully remove supernatant and dispense the cold DNA staining solution (*e.g.,* 5 µM Green Detection Reagent in 1X Assay Buffer) in a volume sufficient for covering the cell monolayer.
5. Protect samples from light and incubate for 30 minutes at 4°C.
6. Flick the staining solution onto a paper towel and if necessary add a few drops of 1X Assay Buffer to prevent the cells from drying out.
7. Cover cells and observe under a fluorescence/confocal microscope with a filter set for GFP/FITC (Ex/Em: 488/514 nm).
8. Positive Control Samples: It is recommended that positive control samples be pre-treated with the Apoptosis Inducer at a final concentration of 2 µM for 4 hours, though optimal conditions may vary significantly among cell types and cell lines. Follow steps 3-7 post treatment.

7.4 Microplate Assay for Chromatin Condensation

The Chromatin Condensation Assay Kit can be employed to analyze chromatin condensation using a fluorescence microplate reader. A 96-well black wall, clear bottom microplate is recommended for this method. Optimal conditions will depend upon the cell type and apoptosis inducer of choice. Fluorescence signals are generally in Relative Fluorescence Units (RFUs) and linearity should be verified with appropriate filter sets according to instrument specifications.

1. Cells should be maintained via standard tissue culture practices
2. Plate 40,000-50,000 cells/100 µL/well in a 96-well black wall, clear bottom plate. Grow overnight.
3. Replace the media with the apoptosis inducer of choice at required concentration and incubate for 4-6 hours at 37°C.
4. Remove the media, then wash with 1X Assay Buffer.
5. Add DNA staining solution (1-5 µM Green Detection Reagent in 1X Assay Buffer).
6. Incubate at 4°C for 30 minutes.
7. Read the plate in the microplate reader with excitation wavelength ~488 nm and emission wavelength ~520 nm.

8. Data Analysis

8.1 Fluorescence Channel Selection for Data collection

The selection of optimal filter sets for a fluorescence microscopy application requires matching the optical filter specifications to the spectral characteristics of the dyes employed in the analysis. Consult the microscope or filter set manufacturer for assistance in selecting optimal filter sets for your microscope. Fluorescence channel FL1 is recommended for imaging Green Detection Reagent with 488 nm excitation.

8.2 Expected results

Apoptosis is characterized by a diverse repertoire of biochemical and cellular signals that alter cellular morphology. Some of the downstream effects observed via fluorescence microscopy include cell shrinkage, loss of membrane symmetry, membrane blebbing and cytoplasmic condensation. Changes at the nuclear level are also evident, including the condensation and fragmentation of nuclear and chromosomal DNA.

8.3 Microscopy

When observed under the fluorescence microscope, cells treated with Staurosporine or other apoptosis inducers should demonstrate stages of chromatin condensation. Nuclei of the healthy cells demonstrate even nuclear staining with distinctly stained nucleoli. Whereas the cells undergoing apoptosis lose the nucleolar staining and the nuclei take up more stain to demonstrate more dense and compact staining pattern.

8.4 Flow Cytometry

a. Positive Control Flow cytometry resolves the healthy cell population from the apoptotic cell population undergoing chromatin condensation based upon an approximately 40-fold increased staining of the condensed chromosomes. Control uninduced Jurkat (T-Cell leukemia) cells are stained as well but mostly display low fluorescence with the

exception of a small percentage of cells (up to 15%) with condensed chromatin, as expected in routine cultures of untreated cells (Figure 2, panels A and C). In the samples treated with 2 µM Staurosporine for 4 hours more than 50% of the cells become highly fluorescent, indicating late-stage apoptosis (Figure 2, panels B and D). Panels A and B display the raw, ungated data. The rectangles in panels A and B are suggested gating settings for flow cytometry. Panels C and D show the data after the suggested gated has been applied, demonstrating clear separation of healthy cells from and apoptotic cells with condensed chromatin (panel D).

b. Appearance of Additional Populations. In addition to the two major populations of weakly (healthy) and strongly (apoptotic) fluorescent cells, additional populations may be resolved based upon the overall granularity and shape size of the cells (as observed by side scatter and/or fluorescence FL1), depending on the nature of the apoptosis inducer. These populations may represent cells undergoing apoptosis at different stages or cells compromised in any other way.

c. Sub-G_0 Analysis. Sub-G_0 analysis of apoptotic cells, a measure of DNA fragmentation, may be performed using the Chromatin Condensation Assay Kit, simply by permeabilizing the cells. The Green Detection Reagent intercalates with the chromosomal DNA and a population of lower fluorescence fragmented DNA can be analyzed as shown in Figure 3.

9. FAQs / Troubleshooting

Problem	Reason	Solution
Assay not working	Assay buffer at wrong temperature	Assay buffer must not be chilled – needs to be at RT
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, protocol will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Ensure you are using appropriate reader and filter settings
	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Sample readings are outside linear range	Concentrate/dilute samples to be in linear range

Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Too many freeze/thaw cycles	Aliquot samples to reduce the number of freeze/thaw cycles
	Samples are too old or incorrectly stored	Use fresh made samples and store at recommended temperature until use
Lower/higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently m ix prior use
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)
Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently m ix prior use
	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes

	Incorrect pipetting when setting up the reaction mix	Always prepare a master mix
Standard curve is not linear	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates

10. Typical Data

Data provided **for demonstration purposes only.**

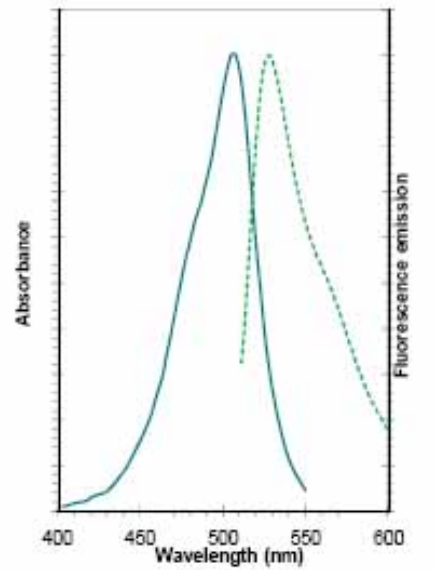


Figure 1: Absorption and fluorescence emission spectra for Green Detection Reagent. Spectra were determined in 1 X Assay Buffer.

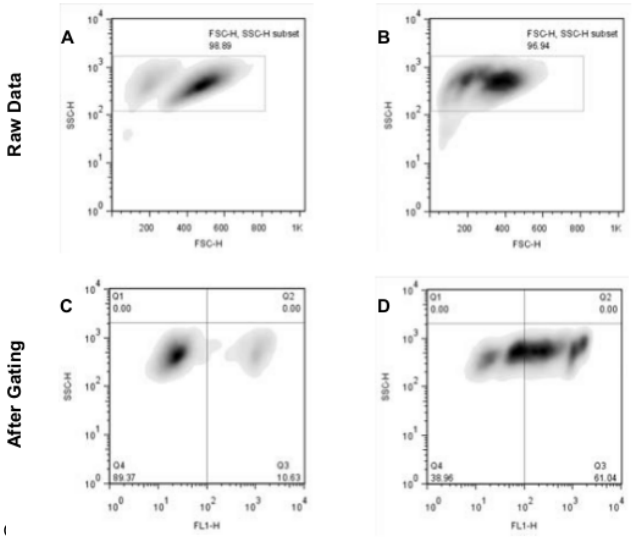
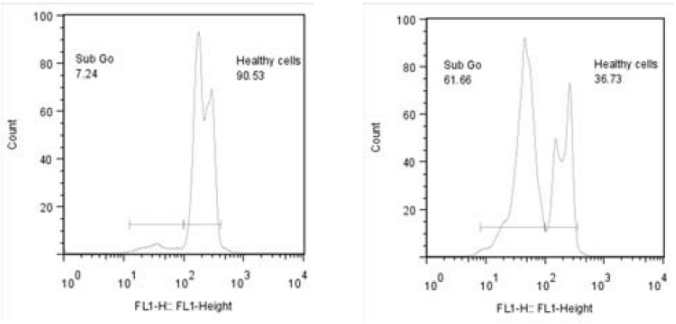


Figure 2: Flow cytometry analysis of Control and Staurosporine-treated Jurkat cells. Raw data is presented in panels A and B where suggested gating is indicated by rectangles drawn around the cell populations. Panels C and D represent the separation of healthy and apoptotic nuclei based on their characteristic fluorescence.



Control **Staurosporine**

Figure 3: Detection of the Sub-G0 phase in permeabilized cells using Green Detection Reagent. In the permeabilized cells, the dye intercalates with the chromosomal DNA, resulting in a highly fluorescent healthy cell population. Upon Staurosporine treatment, a population with lower fluorescence, corresponding to fragmented DNA, can be detected via flow cytometry.

11. Notes

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