

Cellomics[®] Cleaved PARP Detection Kits

For High-Content Screening

1989.0

Number	Description
8402701	Cleaved PARP Detection Kit, sufficient materials for 1 × 96 wells
8402702	Cleaved PARP Detection Kit, sufficient materials for 5 × 96 wells

Kit Contents:	8402701	8402702
Cleaved PARP Primary Antibody	12 µl	60 µl
DyLight™ 549 Conjugated Goat Anti-Mouse IgG	30 µl	72 µl
Hoechst Dye	30 µl	30 µl
Wash Buffer (10X Dulbecco's PBS)	100 ml	100 ml
Wash Buffer II (10X Dulbecco's PBS with 1% Tween-20)	100 ml	100 ml
Permeabilization Buffer (10X Dulbecco's PBS with 1% Triton [®] X-100)	100 ml	100 ml
Blocking Buffer (10X)	85 ml	85 ml
Thin Plate Seal Assembly	7/pack	7/pack

Storage: Upon receipt immediately store the Cleaved PARP Primary Antibody at -20°C. Store all other components at 4°C. Keep vials containing the fluorescent antibody and Hoechst Dye solutions protected from light. Allow buffers to warm to room temperature before use. See the **Solution Preparation** section for storage and stability of prepared solutions.

Warning: Please completely read these instructions and the accompanying material safety data sheets before using this product. The Cellomics Reagents are not for diagnostic use in humans or animals.

Introduction

The Cellomics Cleaved PARP Detection Kit enables detection and quantitation of cleaved PARP in the nuclei. The kit contains a primary monoclonal antibody that detects only the cleaved portion of human PARP, a goat anti-mouse secondary antibody conjugated to DyLight 549 Fluorophore and various other reagents and buffers that are required for immunofluorescence staining for high-content screening (HCS) assays.

Poly (ADP-ribose) polymerase (PARP) cleavage is an important marker of caspase 3-mediated apoptosis. PARP is a 116 kDa nuclear protein involved in repair of DNA nicks induced by various stressors and is one of the substrates for caspase 3, which cleaves PARP into a 85 kDa fragment during apoptosis.^{1,2} In human PARP, cleavage occurs at Asp 214 and Gly 215 leading to formation of 89 and 24 kDa fragments. Cleavage of PARP correlates with DNA fragmentation and other morphological changes making it a critical marker of apoptosis.

Cleaved PARP in HeLa cells treated with staurosporine was quantitatively assayed using the Cellomics Cleaved PARP Detection Kit, Cellomics ArrayScan[®] HCS Reader³ (Figure 1) and the Cellomics Compartmental Analysis BioApplication Software Module. Induction of apoptosis by staurosporine leads to cleavage of PARP and, therefore, increased staining in the nucleus by the anti-cleaved PARP antibody. Cells labeled using this kit also can be imaged by fluorescence or confocal microscopy.

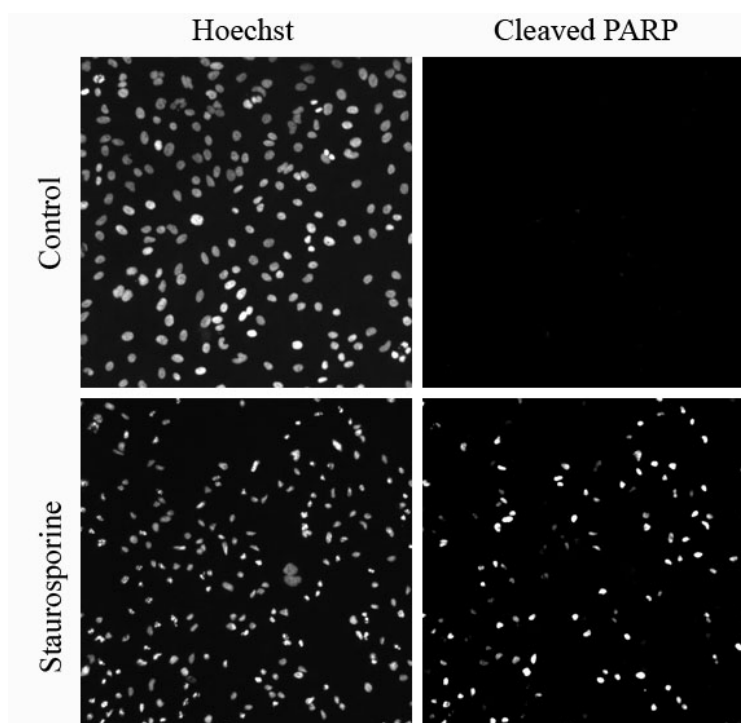


Figure 1. Staining of cleaved PARP in HeLa cells treated with vehicle (0.1% DMSO in media) or with 1 μ M staurosporine for 3 hours. Cells were stained according to the kit protocol and imaged using a Cellomics ArrayScan HCS Reader.

Additional Materials Required

- Thermo Scientific 16% Formaldehyde (Product No. 28906)
- Packard View 96-well microplates (Perkin-Elmer # 6005182)
- Positive control compound, such as staurosporine (Sigma # S3921)
- Fetal bovine serum

Cell Preparation Information

- This protocol is optimized for HeLa cells (American Type Culture Collection #CCL-2). HepG2 and HT1080 cells have been used successfully. Using other cell lines may require optimization.
- For routine culture of cells use EMEM medium containing the following supplements: 10% fetal bovine serum, 1 mM sodium pyruvate, non-essential amino acids and 100 units/ml penicillin, and 100 μ g/ml streptomycin (EMEM complete medium).
- Dilute cells when they reach 90% confluence at a ratio of 1:3. Use cells at a passage number \leq 20.
- Harvest cells by trypsinization, dilute into EMEM complete medium, and determine cell density. Dilute cells to 10^5 cells/ml in EMEM complete medium and add 100 μ l of the cell suspension per well of a 96-well microplate to achieve the recommended plating density of 10,000 cells/well.
- Grow cells overnight at 37°C in 5% CO₂ before drug treatment.

Cleaved PARP Detection Kit Protocol

A. Solution Preparation (per 96-well plate)

1X Wash Buffer	Add 20 ml 10X Wash Buffer to 180 ml ultrapure water for a final volume of 200 ml. Store buffer at 4°C for up to 7 days.
1X Wash Buffer II	Add 6 ml of 10X Wash Buffer II to 54 ml ultrapure water for a final volume of 60 ml. Store buffer at 4°C for up to 7 days.
Fixation Solution	Add 3 ml of 16% Formaldehyde to 9 ml of 1X Wash Buffer just before use.
1X Permeabilization Buffer	Add 1.5 ml of 10X Permeabilization Buffer to 13.5 ml of the 1X Wash Buffer. Store this buffer at 4°C for up to 7 days.
1X Blocking Buffer	Add 5 ml of 10X Blocking Buffer to 44 ml of 1X Wash Buffer and 1 ml of fetal bovine serum for a final volume of 50 ml. Store this buffer at 4°C for up to 7 days.
Primary Antibody Solution	Add 12 µl of primary antibody to 6 ml of 1X Blocking Buffer without FBS. Prepare solution just before each assay.
Secondary Antibody/Staining Solution	Add 0.6 µl of Hoechst Dye and 12 µl of the DyLight 549 Goat Anti-Mouse Antibody to 6 ml of 1X Blocking Buffer without FBS. Prepare solution just before each assay.

B. Procedure

1. Prepare 2X solution of staurosporine (2 µM), add 100 µl to the cells and incubate for 3 hours at 37°C.
2. Aspirate culture medium and add 100 µl of Fixation Solution to each well. Incubate plate in a fume hood at room temperature for 15 minutes.
3. Aspirate Fixation Solution, and wash plate twice with 100 µl/well of 1X Wash Buffer.
4. Aspirate Wash Buffer, add 100 µl/well of 1X Permeabilization Buffer and incubate for 15 minutes at room temperature.
5. Aspirate Permeabilization Buffer and wash plate twice with 100 µl/well of 1X Wash Buffer.
6. Aspirate Wash Buffer, add 100 µl/well of 1X Blocking Buffer supplemented with 2% fetal bovine serum (FBS) and incubate at room temperature for 15 minutes.
7. Aspirate Blocking Buffer and add 50 µl/well of Primary Antibody Solution. Incubate for 1 hour at room temperature.
8. Aspirate Primary Antibody Solution and wash plate twice with 100 µl/well of 1X Wash Buffer II.
9. Aspirate Wash Buffer II and wash plate once with 100 µl/well of 1X Wash Buffer.
10. Aspirate wash buffer and add 50 µl/well of Secondary Antibody/Staining Solution. Incubate for 45 minutes protected from light at room temperature.
11. Aspirate Staining Solution and wash plate twice with 100 µl/well of 1X Wash Buffer II.
12. Aspirate Wash Buffer II and wash plate twice with 100 µl/well of 1X Wash Buffer.
13. Aspirate Wash Buffer and replace with 200 µl/well of 1X Wash Buffer.
14. Seal plate and evaluate on the ArrayScan HCS Reader or by fluorescence microscopy. Store plates at 4°C.

Additional Information

A. Dose Response Curve

HeLa cells were treated with different doses of staurosporine for 3 hours as described in the procedure, and intensity of the cleaved PARP was measured (Figure 2).

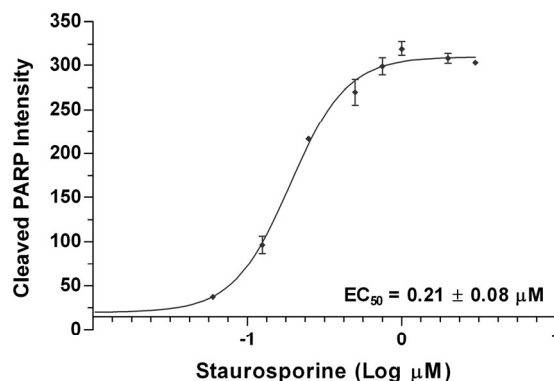


Figure 2. Dose response curve for cleaved PARP intensity in treated HeLa Cells. Wells were scored using the output parameter of the average nuclear intensity of cleaved PARP intensity (Mean_CircAvgInt). Data represents mean \pm SD from three plates (eight wells per 96-well plate per dose of staurosporine).

B. Performance Robustness

Assay robustness was ascertained by determining the Z' for the total intensity of cleaved PARP in non-treated cells (vehicle) and cells treated with staurosporine (1 μM for 3 hours).⁴ The mean \pm SD of the Z' was calculated using three plates of HeLa cells treated identically and was 0.540 ± 0.004 .

DMSO tolerance: The assay performance using these kits was robust when compounds were added in DMSO up to a maximum concentration of 1% DMSO.

C. Microscope Information

Cells prepared and labeled according to these instructions can be used and analyzed by fluorescence microscopes using the appropriate filter set(s) or confocal microscopy. Optimization may be required when using slides, coverslips or multi-well chamber slides. Use image-processing software to quantify the targets. The approximate absorption/emission maxima of the fluorescent dyes are as follows:

DyLight 549 Conjugates = 550/568 nm

Hoechst Dye = 350/461 nm

D. Recommendations for Automation

- **Plating Cells:** To improve the uniformity and throughput of plating cells, use a liquid handling system such as Thermo Scientific Multidrop[®] Combi or WellMate[®] Dispensers.
- **Dead Volumes:** Every piece of automation instrumentation has a non-recoverable dead volume associated with it. Be aware of these dead volumes, priming volumes and rinsing volumes when calculating your reagent requirements.
- **Nonspecific Binding:** Because of the potential of reagent interaction with large surface areas inherent to tubing, syringes and peristaltic pumps, pre-priming with reagents or pre-coating with protein blockers may be warranted.
- **Mixing:** Gentle mixing may be required when adding a DMSO-based solution to keep overly concentrated solutions from lying on top of the cell layer. Be careful not to dislodge cells or beads during mixing procedures.
- **Cell Washing:** Use an automated plate washer designed to gently wash attached cells. Be careful not to dislodge cells or beads during cell washing.

- Incubation: Minimize the time when plates with live cells are out of a controlled CO₂ environment. For best results, use an automated incubator to deliver plates to a pipetting deck.
- Exposure: Minimize operator exposure to fixative by some form of containment. Some reagents and compounds are light-sensitive; be aware of these constraints when scaling up for an automated run.
- Adapting to other plate formats: When using different plate types, adjust reagent volumes as needed. Some suggested starting volumes are listed in Table 1.

Table 1. Suggested volumes to use for different cell culture plates.

<u>Kit Component</u>	<u>96-Well Plates</u> (<u>µl/well</u>)	<u>384-Well Plates</u> (<u>µl/well</u>)	<u>24-Well Plates</u> (<u>µl/well</u>)
Fixation Solution	100	25	400
1X Wash Buffer	100	25	400
Wash Buffer II	100	25	400
1X Permeabilization Buffer	100	25	400
1X Blocking Buffer	100	25	400
Antibody Solution	50	12.5	200
Staining Solution	50	12.5	200
1X Wash Buffer (final wash)	150	37.5	200

Compatible BioApplication Software Modules

S50-0001-1 or S50-2001-1	Cytoplasm to Nucleus Translocation BioApplication
S50-5019-1 or S50-2019-1	Molecular Translocation BioApplication
S50-5011-1 or S50-2011-1	Target Detection BioApplication
S50-5017-1 or S50-2017-1	Compartmental Analysis BioApplication

References

1. Tewari, M., *et al.* (1995). YAMA/CPP32 beta, a mammalian homolog of CED-3, is a CrmA-inhibitable protease that cleaves the death substrate poly(ADP-ribose) polymerase. *Cell* **81**(5):801-9.
2. Nicholson, D.W., *et al.* (1995). Identification and inhibition of the ICE/CED-3 protease necessary for mammalian apoptosis. *Nature* **376**:37-43.
3. Taylor, D.L., *et al.* (2007). High content screening: A powerful approach to systems cell biology and drug discovery. *Method Mol Biol* **356**. Humana Press, Totowa, N.J.
4. Zhang, J.H., *et al.* (1999). A simple statistical parameter for use in evaluation and validation of high throughput screening assays. *J Biomol Screen* **4**:67-73.

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