# Biocolor's APOPercentage Apoptosis Assay™ on BMG LABTECH's Spectrostar *Nano* plate reader

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- Monitors occurrence of apoptosis in live, mammalian, anchorage-dependent cells
- Detects and quantifies percentage of apoptosis in cell population

## Introduction

Apoptosis is a multistage process during which activity of caspase enzymes fluctuates, DNA becomes fragmented and phosphatidyl serine is transferred to the outside of the cell membrane.

***Common methods for analysis include:***

1. Caspase activity assay, either colorimetric, fluorescent, luminescent or antibody based.
2. TUNEL assay, based on DNA fragmentation.
3. Annexin-V assay, based on the binding of dye or fluorescently-conjugated Annexin-V to phosphatidyl serine which has translocated to the cell membrane exterior during apoptosis.

Necrotic cells must be distinguished using propidium iodide.

***APOPercentage Principle***[1]

Transfer and exposure of phosphatidyl serine to the exterior surface of the cell membrane has been linked to the onset of apoptosis. Phosphatidyl serine transmembrane movement results in the uptake of APOPercentage Dye by apoptotic committed cells. Dye uptake continues until blebbing of the apoptotic committed cell occurs. No further dye can then enter the defunct cell and the dye that has accumulated within the cell is not released. Necrotic cells do not retain the dye. Dyed cells can be counted using a light microscope or the dye can be released from the cells and measured spectrophotometrically as in the method below.

## Materials and Methods

This application note describes a simple colorimetric method for the detection and quantification of apoptosis. The assay requires:

- ☐ Minimum cell culture facilities
- ☐ BMG LABTECH SPECTROstar *Nano* plate reader (Fig. 1)
- ☐ 24 and 96 well microplates

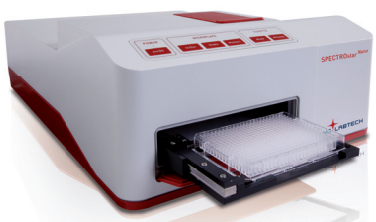

**Fig. 1:** BMG LABTECH's microplate reader SPECTROstar *Nano*.

***Protocol***

1. Seed a 24-well tissue culture plate with 5 x $10^4$ cells/well in 500 µL culture medium and incubate the cells at 37°C/5% $CO_2$, until confluence is reached (~ 24 h).
2. Prepare dilutions of test apoptotic agent(s) at selected concentrations using the suggested layout below. Controls (-ve & +ve) should be included with each experiment.

| Reag. Blank | Reag. Blank | -ve Control | -ve Control | +ve Control | +ve Control |
|---|---|---|---|---|---|
| Sample 1 | Sample 1 | Sample 4 | Sample 4 | Sample 7 | Sample 7 |
| Sample 2 | Sample 2 | Sample 5 | Sample 5 | Sample 8 | Sample 8 |
| Sample 3 | Sample 3 | Sample 6 | Sample 6 | Sample 9 | Sample 9 |

3. Make up double quantity of **Reagent A.** Use half the volume to prepare **Reagent B**.

| | Reagent A (500µL/well) | Reagent B (500µL/well) |
|---|---|---|
| **Reagent Blank** | Culture medium / serum | Reagent A |
| **Negative Control (0% apoptosis)** | Culture medium / serum | Reagent A + 5% v/v dye |
| **Positive Control (100% apoptosis)** | Culture medium / serum + reference apoptotic agent | Reagent A + 5% v/v dye |
| **Test Samples (›0%, ‹100%)** | Culture medium / serum + test apoptotic agent | Reagent A + 5% v/v dye |

4. Remove the culture medium from each well of the incubated plate and add 500 µL of **Reagent A**, supplemented v/v with serum (if required), to all wells.
5. Incubation time for apoptotic inducer/inhibitor will depend on apoptotic agent used. 30 min before this time period is reached remove **Reagent A**. Immediately replace with 500 µL **Reagent B** and incubate for the remaining 30 min, at 37°C/5% $CO_2$.
6. Remove **Reagent B** from each well using a pipette. Gently wash the cells twice with 1000 µL/well PBS to remove non-cell bound dye. (NOTE: Careful aspiration is advised as some apoptotic agents can cause detachment and loss of cells).
7. Add trypsin (50 µL) to each well and incubate for 10 minutes at 37°C/5% $CO_2$. Tap the plate gently by hand after 5 minutes and

again after 10 minutes to detach cells from the plastic, cell culture treated surface.

8. Now add 200 µL Dye Release Reagent to each well and shake plate for 10 minutes.

9. Transfer contents of each well (250 µL) to a transparent 96 well flat bottom plate and read absorbance at 550 nm using the BMG LABTECH SPECTROstar *Nano*. Bubbles in wells affect results, burst with clean pin or transfer 200 µL instead of 250 µL to microplate.

This experiment was carried out with triplicate samples.

***SPECTROstar Nano Settings***

| | |
|---|---|
| Measurement Type: | Absorbance |
| No. of Flashes per well: | 22 |
| Excitation: | 550 nm |
| Shaking Frequency (rpm): | 300 |
| Shaking Mode: | double orbital |
| Additional Shaking Time: | 3s before plate reading |
| Positioning Delay (s): | 0.2 |

## Results and Discussion

1. Follow protocol as above to obtain absorption data.
2. Subtract the mean value of reagent blank replicates from test results.
3. Plot mean absorbance values ± standard error of the mean in a bar chart (Fig. 2) or as a percentage of the Positive Control absorbance value.

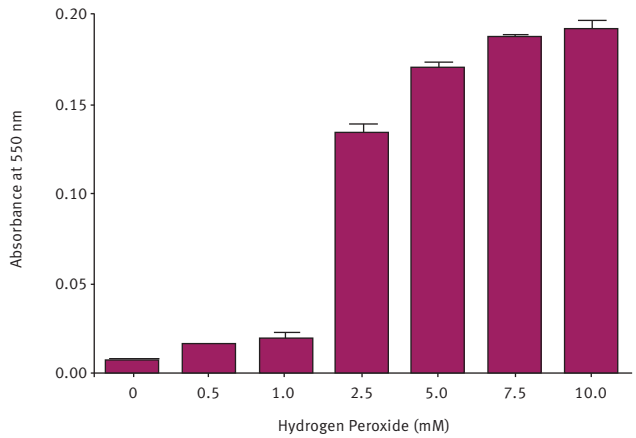


**Fig. 2:** ***Colorimetric Quantification.*** Graph Showing Effect of Hydrogen Peroxide (0 – 10mM) on CHO Cells. Results expressed as mean absorbance for triplicate wells ± S.E.M. (n = 3). Exposure time to $H_2O_2$ was 4 hours.

## Conclusion

The SPECTROstar *Nano* provides a fast, accurate and consistent method for quantifying apoptosis colorimetrically.

## References

1 www.biocolor.co.uk/manuals/apopercentage.pdf

## Background

Biocolor is a UK company based in Carrickfergus, N. Ireland, with a network of distributors throughout the world. Biocolor's expertise lies in its unique range of extracellular matrix assays for use with mammalian cells, tissues and fluids: Sircol™- Collagen, Blyscan™- Glycosaminoglycan and Fastin™-Elastin. VolCol is Biocolor's range of bovine and rat collagen products.

More information can be found at www.biocolor.co.uk


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