

# Detection of Apoptosis in Primary Cells by Annexin V Binding Using the Agilent 2100 Bioanalyzer

## Application Note

Samuel D. H. Chan

Marc Valer and Tobias Preckel, *Agilent Technologies*

### Introduction

The Agilent 2100 bioanalyzer has been used previously to evaluate apoptosis by monitoring annexin V-binding and active caspase-3 in cultured mammalian cell lines<sup>[1]</sup>. A simplified and faster annexin V-binding assay protocol has since been developed. This new protocol has the advantages of shorter total analysis time and lower cell consumption<sup>[2]</sup>.

Therefore, this approach is particularly suited for analysis with limited cell availability, such as primary cells. This application note describes the analysis of apoptosis in primary human endothelial cells and human dermal fibroblasts using the fast annexin V-binding assay protocol together with the Agilent 2100 bioanalyzer.



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## Experimental

### **Reagents and chemicals**

Staurosporine is a product of Sigma Chemical, Corp. (St. Louis, MO, USA). Calcein-AM was purchased from Molecular Probes, Inc./Invitrogen (Eugene, OR, USA). Annexin V-Biotin Apoptosis Detection Kit was obtained from Oncogene Research Products/Calbiochem (La Jolla, CA, USA). Fluorolink Cy5-streptavidin was purchased from Amersham Biosciences (Piscataway, NJ, USA). Cell Fluorescence LabChip Kit and 2100 bioanalyzer are from Agilent Technologies (Waldbronn, Germany)

### **Primary cell culture**

Human umbilical vein endothelial cells (HUVEC), normal human dermal fibroblasts (NHDF), culture media, and trypsin/EDTA solution were obtained from Cambrex Corp. /Clonetics (East Rutherford, NJ, USA). HUVEC were maintained in EGM-2 medium and NHDF were cultured in FGM-2 medium.

### **Induction of apoptosis and annexin-V binding assay**

HUVEC or NHDF were treated with staurosporine for 5 hours to induce apoptosis<sup>[3]</sup>. Treated cells were harvested by trypsinization according to the supplier's protocol and resuspended in culture medium to a cell density of  $1 \times 10^3$  cells/ $\mu$ L. 100  $\mu$ L of the cell suspension were mixed with 2  $\mu$ L of 200  $\mu$ g/mL annexin V-biotin (Annexin V Biotin Apoptosis Detection Kit) in a microcentrifuge tube and incubated for 10 min at room temperature. The medium was centrifuged and the supernatant was aspirated. The cells were resuspended by gentle vortexing in 100  $\mu$ L of 1x Binding Buffer (Annexin V Biotin Apoptosis Detection Kit) containing 1  $\mu$ g/mL Fluorolink Cy5-streptavidin and 1  $\mu$ M Calcein-AM.

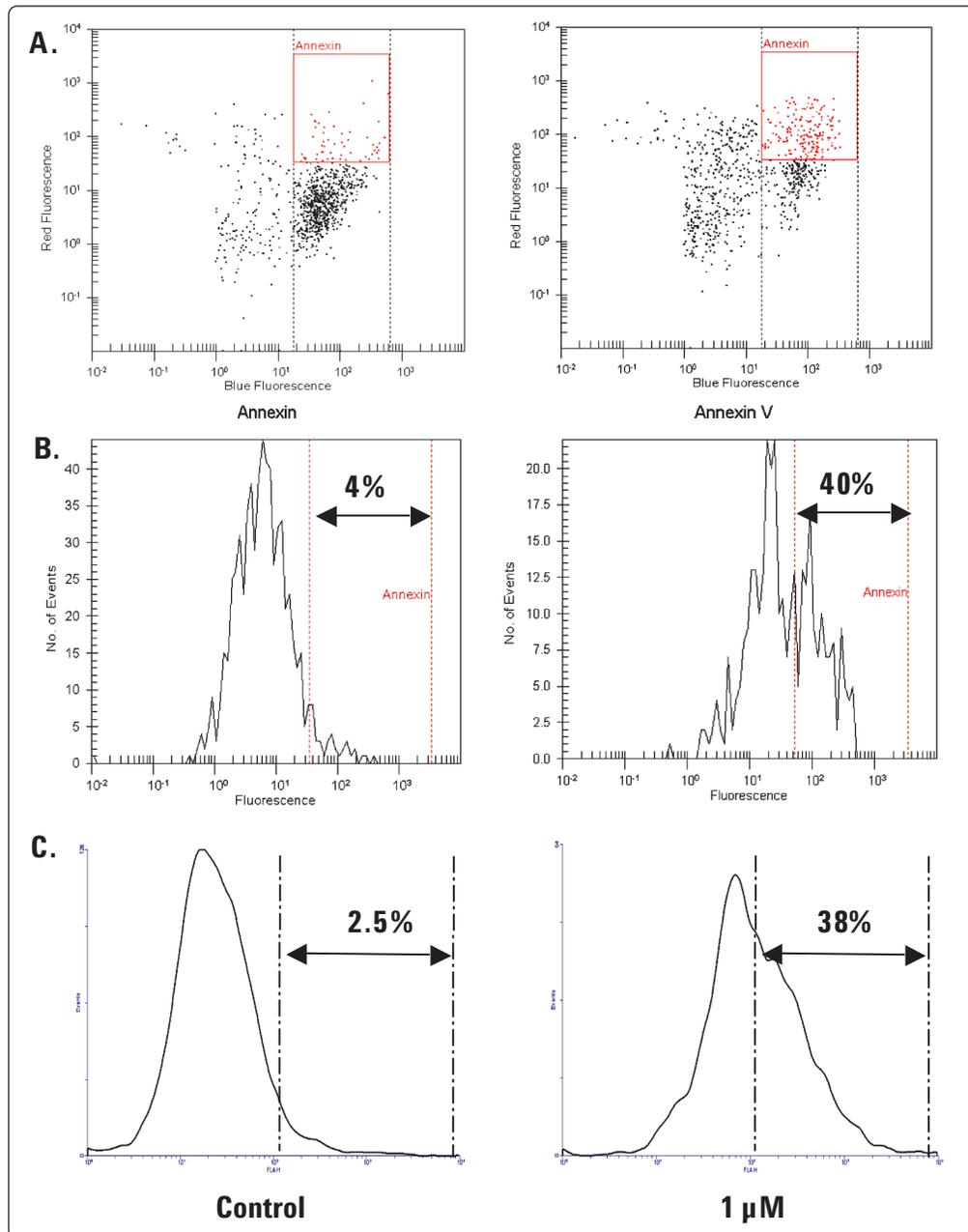
Calcein was used as an indicator for cells with an intact cell membrane. The non-fluorescent AM-ester freely diffuses into cells where it is cleaved by nonspecific esterases into fluorescent products. These rapidly leak out from cells with damaged cell membranes, but accumulate in intact cells.

After incubation for 10 min at room temperature, centrifugation, and medium aspiration, cells were resuspended in 50  $\mu$ L of CB (Cell Buffer, Cell Fluorescence LabChip kit) by gentle pipetting. 10  $\mu$ L of cell suspension (20,000 cells) were added directly to the wells of a cell chip without further treatment or washes. The prepared chip was then loaded on the Agilent 2100 bioanalyzer for analysis.

## Results and Discussion

Annexin V-binding was used to evaluate staurosporine-induced apoptosis in HUVEC and NHDF. The cells were stained using the fast apoptosis assay protocol<sup>[2]</sup> and run on an Agilent 2100 bioanalyzer. For comparison, the cells were also analyzed on a FACSCalibur flow cytometer (BD Biosciences).

Figure 1 shows the results obtained when HUVEC were treated with 1  $\mu$ M staurosporine for 5 hours versus a control sample. Figure 1A and 1B represent the dot plots and frequency histogram plots of the 2100 bioanalyzer assay. 40% of the treated HUVEC were determined to be apoptotic.



**Figure 1.** HUVEC were treated with 1  $\mu$ M staurosporine for 5 hours to induce apoptosis. Control and treated cells were harvested, stained according to the fast apoptosis assay protocol, and then analyzed on the 2100 bioanalyzer (A & B). The numbers represent the percentage of apoptotic cells in the live cell population. Aliquots of the same samples were evaluated on a conventional flow cytometer (C). Approximately 500 cell events were analyzed per sample on the 2100 bioanalyzer whereas 5,000 events were acquired on the flow cytometer, giving equivalent results.

Results from the analysis of NHDF are shown in Figure 2. Approximately 18% of the live, calcein-positive, cells had undergone apoptosis after treatment with 0.5  $\mu\text{M}$  of staurosporine while 5% apoptotic cells were detected in the untreated control sample (Figure 2A, 2B). When the staurosporine concentration was increased to 1  $\mu\text{M}$ , 31% of the cells were found to be apoptotic. Comparable results were obtained when the cells were analyzed on a conventional flow cytometer (Figure 1C, 2C).

## Conclusion

The Agilent 2100 bioanalyzer, in conjunction with the fast annexin V-binding assay protocol, is particularly well suited for the detection of apoptosis in primary cells. The main advantages of this approach are the low cell consumption and minimal cell loss due to the elimination of washing and aspiration steps in the staining protocol and the overall minimal volumes required for microfluidic analysis.

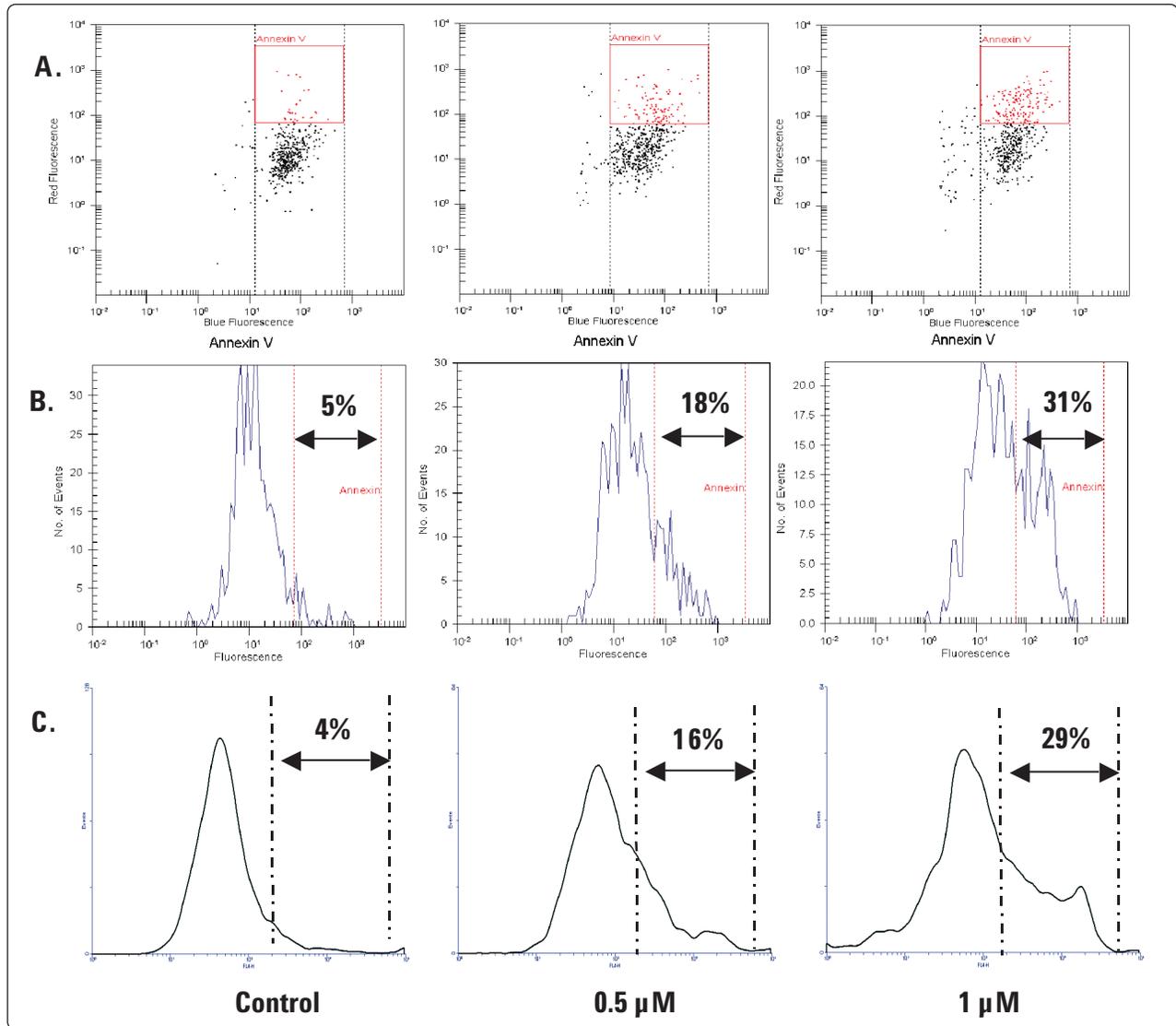


Figure 2. NHDF were treated with 0.5 or 1  $\mu\text{M}$  staurosporine for 5 hours to induce apoptosis. Cells were stained using the fast apoptosis assay protocol and analyzed on the 2100 bioanalyzer (A & B). Panel C shows comparable histograms obtained after analysis using a conventional flow cytometer.

## References

1. Preckel, T. and Luedke, G. (2001). Apoptosis detection by annexin V and active caspase-3 with the Agilent 2100 bioanalyzer. Agilent Application Note 5988-4322EN.
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## Authors

**Samuel D. H. Chan** is a Research Consultant in Daly City, CA, USA.

**Marc Valer** and **Tobias Preckel** are Product Managers at Agilent Technologies R&D and Marketing GmbH & Co. KG., Waldbronn, Germany.

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