pSIVA™ REAL-TIME APOPTOSIS FLUORESCENT MICROSCOPY KIT

(APO004)

Kit Components

Component	Recommended Storage Temperature	Quantity	Excitation Maximum, nm	Emission Maximum, nm	Recommended Dilution Range
pSIVA™-IANBD	4°C (Do not freeze)	200 µl	488	530	10-20 µl/ml
Propidium Iodide Staining Solution	4°C (Do not freeze)	500 µl	535	617	5-10 µl/ml

BIO-RAD

Basic Fluorescence Microscopy Protocol using pSIVA Real-Time Apoptosis Fluorescent Microscopy Kit

Prior to commencing the microscopy experiment, please ensure that your cell culture medium contains between 1-2 mM Ca^{2+}. Ca^{2+} is essential for binding of the pSIVA-IANBD probe to exposed phosphatidylserine (Kim et al. 2010). If Ca^{2+} levels are insufficient, supplement the culture medium with 2 mM Ca^{2+}.

Step 1 **Seed cells into culture plates and allow cells to adhere*[1].**

Step 2 (optional) **After 24 hours exchange the culture medium for medium containing 2 mM Ca^{2+} (if required).** For best imaging results, reduce autofluorescence from culture medium by using low fluorescence media.

Step 3 (optional) **Induce apoptosis by treating cells with apoptosis inducing agents such as staurosporine or camptothecin.** Consult scientific literature and protocols to determine the optimal treatment conditions. We recommend including negative controls for all treatment conditions in your experimental design (see controls section).

Step 4 **Add 10–20 µl/ml*[2] of the pSIVA-IANBD probe to cells.** Mix gently by moving culture plates backwards and forwards and side to side to ensure even distribution of the probe. DO NOT PIPETTE TO MIX.

Step 5 (optional) **If distinction between apoptotic and necrotic/dead cells is desired, add between 5–10 µl/ml*[2] of propidium iodide (PI) to cells.** Mix gently by moving plates backwards and forwards and side to side to ensure even distribution of PI. DO NOT PIPETTE TO MIX.

Step 6 **Observe cells under microscope using the green fluorescence filter for pSIVA-IANBD (excitation maximum 488 nm, emission maximum 530 nm) and the red fluorescence filter for PI (excitation maximum 535 nm, emission maximum 617 nm) visualization.** Staining might be observed immediately. However, it can take up to 10 minutes before staining is detected. Staining may also change over time.

*[1] Please note that this protocol has been developed for use with adherent mammalian cell lines only.
*[2] The stated pSIVA-IANBD and PI quantities are guidelines only and may have to be optimized.

Controls

- Include unstained cells (i.e. no addition of pSIVA-IANBD or PI) as negative controls.
- Include stained untreated cells as negative controls. In most cases the solvent used for making up the apoptosis inducing agent should be added to control for the effects of the solvent.
- Positive control cells, such as HeLa cells treated with 1 µM staurosporine, may be included (see Figure 1).

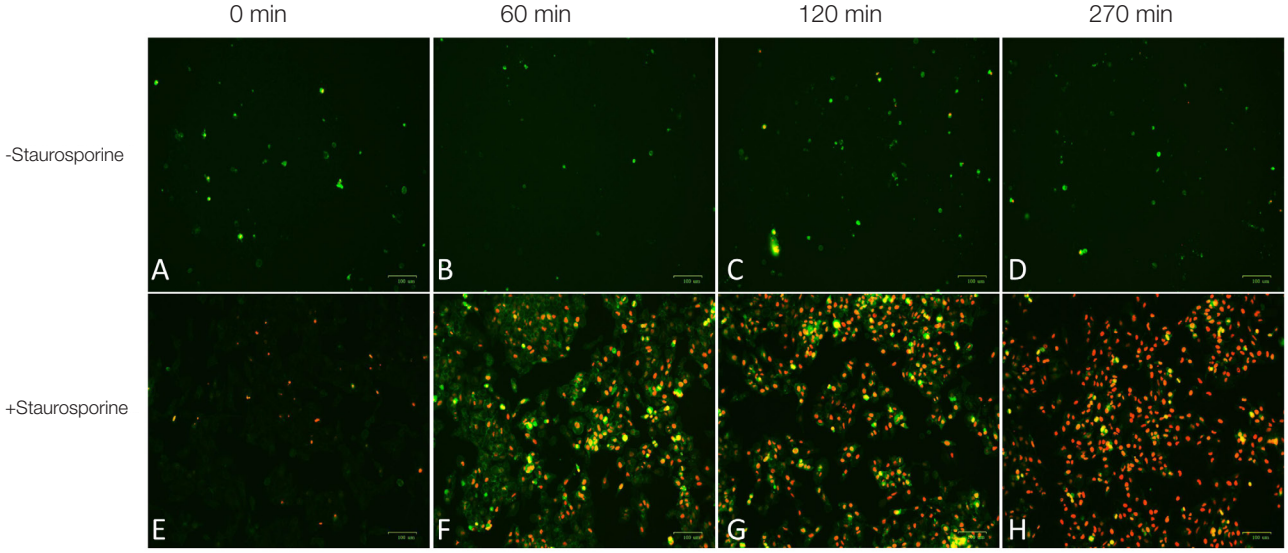
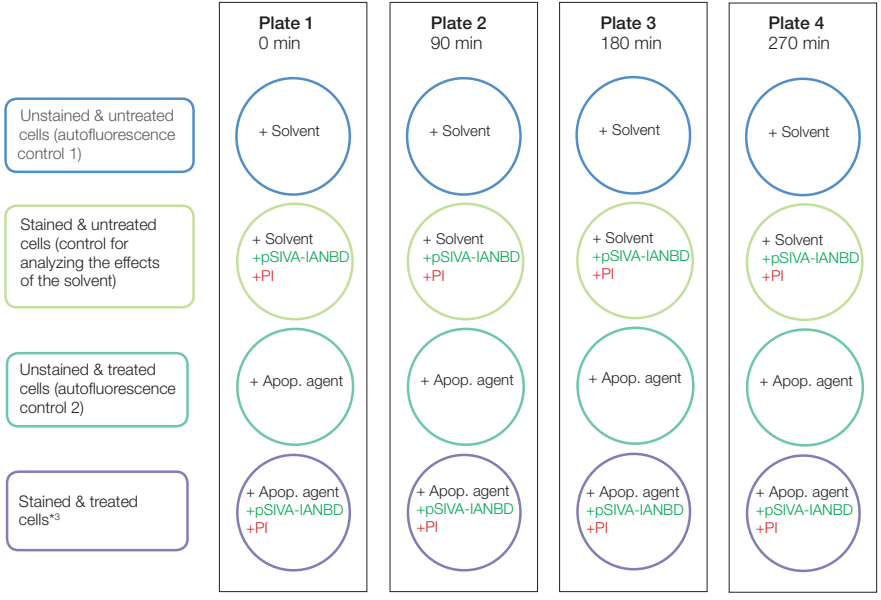


Fig. 1. 1x10^5 HeLa cells were seeded on Millicell® EZ Slide 4-well glass slides (sterile) and cultured in 0.5 ml RPMI medium supplemented with 2 mM $CaCl_2$. Cells were treated with 1 µM of the apoptosis inducing agent staurosporine (Figures E, F, G, H) or left untreated (Figures A, B, C, D). pSIVA–IANBD + PI were added to the media according to kit instructions. Cells were imaged post treatment with the ZOE™ Fluorescent Cell Imager at 0 minutes, 60 minutes, 120 minutes and 270 minutes. Images A-H are merged images of the red and green fluorescence channels. Early apoptotic cells were stained with pSIVA-IANBD and are shown in green while late apoptotic/dead cells were stained with PI and are shown in red.

Experimental design overview example



Abbreviations: Apop. agent, apoptotic agent; Solvent, solvent used to make up the apoptotic agent

*3 For certain experiments other positive controls may be included (e.g. staining of different cell types)

References

Kim et al. (2010). Monitoring apoptosis and neuronal degeneration by real-time detection of phosphatidylserine externalization using a polarity-sensitive indicator of viability and apoptosis. Nature Protocols, 5:1396-1405.

BIO-RAD

Bio-Rad Laboratories, Inc.

Life Science Group

Web site *www.bio-rad-antibodies.com*
Americas Toll free *1 800 265 7376 (USA & Canada)* ***Americas*** *1 919 878 7978* ***Email:*** *antibody_sales_us@bio-rad.com*
Europe *49 89 80 90 95 21* ***Email:*** *antibody_sales_de@bio-rad.com*
UK & Worldwide *44 1865 852700* ***Email:*** *antibody_sales_uk@bio-rad.com*

LIT.APO.V1.0316