

## Datasheet: APO004

**BATCH NUMBER 158313**

<b>Description:</b>	pSIVA™ REAL-TIME APOPTOSIS FLUORESCENT MICROSCOPY KIT
<b>Name:</b>	pSIVA™ MICROSCOPY KIT
<b>Other names:</b>	ANNEXIN 12, ANNEXIN XII
<b>Format:</b>	IANBD (Green Fluorescence)
<b>Product Type:</b>	Kits
<b>Quantity:</b>	1 KIT

## Product Details

### Applications

This product has been reported to work in the following applications. This information is derived from testing within our laboratories, peer-reviewed publications or personal communications from the originators. Please refer to references indicated for further information. For general protocol recommendations, please visit [www.bio-rad-antibodies.com/protocols](http://www.bio-rad-antibodies.com/protocols).

	Yes	No	Not Determined	Suggested Dilution
Immunofluorescence	▪			Refer to Instructions For Use
Immunocytochemistry	▪			Refer to Instructions For Use
Live Cell Imaging	▪			Refer to Instructions For Use

Where this product has not been tested for use in a particular technique this does not necessarily exclude its use in such procedures. Suggested working dilutions are given as a guide only. It is recommended that the user titrates the product for use in their own system using appropriate negative/positive controls.

### Product Information

The process of apoptosis is undertaken in several stages defined by specific cellular morphologies. One of the earlier stages of apoptosis is a change of the plasma membrane's phospholipid asymmetry. This rearrangement results in the translocation of phosphatidylserine (PS) from the inner to the outer plasma membrane (in non-apoptotic cells PS is exclusively located to the inner plasma membrane). However, apoptosis is reversible until reaching a certain point in the pathway and until then PS exposure can be considered as a transient event. The event defining whether the cell can be rescued and continues living is the onset of mitochondrial outer membrane permeabilization (Chipuk *et al.* 2006). Prior to reaching this point, PS exposure may be transient as molecules can relocate back to the inner plasma membrane (a phenomenon known as "PS flipping") ([van der Mark \*et al.\* 2013](#)).

The pSIVA™ (polarity-Sensitive Indicator of Viability & Apoptosis) probe is a biosensor conjugated to the green emitting IANBD dye (excitation maximum 488 nm, emission

maximum 530 nm) and only fluoresces when bound to PS in the presence of  $\text{Ca}^{2+}$  (Kim *et al.* 2010a, 2010b). The method thereby allows the analysis of kinetic apoptosis events in real time by live cell imaging and immunofluorescence / immunocytochemistry. In contrast to other PS detection based assays (e.g. annexin V) the pSIVA™ Real-Time Apoptosis Fluorescent Microscopy Kit does not require washing steps as you can simply add the probe and start analyzing.

<b>Reagents In The Kit</b>	pSIVA-IANBD 200 $\mu\text{l}$ Propidium Iodide Staining Solution 500 $\mu\text{l}$
<b>Instructions For Use</b>	<p>Prior to commencing the microscopy experiment, please ensure that your cell culture medium contains between 1-2 mM <math>\text{Ca}^{2+}</math>. <math>\text{Ca}^{2+}</math> is essential for binding of the pSIVA-IANBD probe to exposed phosphatidylserine (Kim <i>et al.</i> 2010b). If <math>\text{Ca}^{2+}</math> levels are insufficient, supplement the culture medium with 2 mM <math>\text{Ca}^{2+}</math>.</p> <ol style="list-style-type: none"> <li>1. Seed cells into culture plates and allow cells to adhere.</li> <li>2. Optional. After 24 hours exchange the culture medium for medium containing 2 mM <math>\text{Ca}^{2+}</math>, if required.</li> <li>3. Optional. Induce apoptosis by treating cells with apoptosis inducing agents such as staurosporine or camptothecin.</li> <li>4. Add 10–20 <math>\mu\text{l}/\text{ml}^*</math> 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. <b>DO NOT PIPETTE TO MIX.</b></li> <li>5. Optional. If distinction between apoptotic and necrotic/dead cells is desired, add between 5–10 <math>\mu\text{l}/\text{ml}^*</math> of propidium iodide (PI) to cells. Mix gently by moving plates backwards and forwards and side to side to ensure even distribution of PI. <b>DO NOT PIPETTE TO MIX.</b></li> <li>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.</li> </ol> <p>* The stated pSIVA-IANBD and PI quantities are guidelines only and may have to be optimized.</p> <p>Instructions for use can be found at <a href="http://www.bio-rad-antibodies.com/uploads/IFU/APO004.pdf">www.bio-rad-antibodies.com/uploads/IFU/APO004.pdf</a></p>
<b>References</b>	<ol style="list-style-type: none"> <li>1. Kim, Y.E. <i>et al.</i> (2010) (a) Engineering a polarity-sensitive biosensor for time-lapse imaging of apoptotic processes and degeneration. <a href="#">Nat Methods 7(1): 67–73.</a></li> <li>2. Kim, Y.E. <i>et al.</i> (2010) (b) Monitoring apoptosis and neuronal degeneration by real-time detection of phosphatidylserine externalization using a polarity-sensitive indicator of viability and apoptosis. <a href="#">Nat Protoc. 5(8): 1396-405.</a></li> </ol>
<b>Storage</b>	<p>Store at +4°C. DO NOT FREEZE.</p> <p>This product should be stored undiluted. This product is photosensitive and should be protected from light.</p>
<b>Guarantee</b>	6 months from date of despatch

**Acknowledgements** pSIVA™ is a trademark of Novus Biologicals and is protected under patent no. 8.541.549.

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**Health And Safety Information** Material Safety Datasheet documentation #10587 #10588 available at:  
<https://www.bio-rad-antibodies.com/SDS/APO004>  
Propidium Iodide Staining Solution (10587)  
pSIVA-IANBD (10588)

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**Regulatory** For research purposes only

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## Related Products

### Recommended Useful Reagents

[ANNEXIN V:PE ASSAY KIT \(ANNEX50PE\)](#)

[ANNEXIN V:PE ASSAY KIT \(ANNEX200PE\)](#)

[ANNEXIN V:APC ASSAY KIT \(ANNEX50APC\)](#)

[ANNEXIN V:APC ASSAY KIT \(ANNEX200APC\)](#)

**North & South** Tel: +1 800 265 7376

**America** Fax: +1 919 878 3751

Email: [antibody\\_sales\\_us@bio-rad.com](mailto:antibody_sales_us@bio-rad.com)

**Worldwide**

Tel: +44 (0)1865 852 700

Fax: +44 (0)1865 852 739

Email: [antibody\\_sales\\_uk@bio-rad.com](mailto:antibody_sales_uk@bio-rad.com)

**Europe**

Tel: +49 (0) 89 8090 95 21

Fax: +49 (0) 89 8090 95 50

Email: [antibody\\_sales\\_de@bio-rad.com](mailto:antibody_sales_de@bio-rad.com)

To find a batch/lot specific datasheet for this product, please use our online search tool at: [bio-rad-antibodies.com/datasheets](https://www.bio-rad-antibodies.com/datasheets)  
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