

Datasheet: APO004

**BATCH NUMBER 167163**

<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 Ca<sup>2+</sup> ([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.

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**Reagents In The Kit** pSIVA-IANBD 200 µl  
Propidium Iodide Staining Solution 500 µl

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**Instructions For Use** Prior to commencing the microscopy experiment, please ensure that your cell culture medium contains between 1-2 mM Ca<sup>2+</sup>. Ca<sup>2+</sup> is essential for binding of the pSIVA-IANBD probe to exposed phosphatidylserine ([Kim et al. 2010b](#)). If Ca<sup>2+</sup> levels are insufficient, supplement the culture medium with 2 mM Ca<sup>2+</sup>.

1. Seed cells into culture plates and allow cells to adhere.
  2. Optional. After 24 hours exchange the culture medium for medium containing 2 mM Ca<sup>2+</sup>, if required.
  3. Optional. Induce apoptosis by treating cells with apoptosis inducing agents such as staurosporine or camptothecin.
  4. Add 10–20 µl/ml\* 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.**
  5. Optional. If distinction between apoptotic and necrotic/dead cells is desired, add between 5–10 µl/ml\* 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.**
  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.
- \* The stated pSIVA-IANBD and PI quantities are guidelines only and may have to be optimized.

[pSIVA Real-time Apoptosis Fluorescent Microscopy Kit](#) Instructions.

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**References**

1. Kim, Y.E. *et al.* (2010) (a) Engineering a polarity-sensitive biosensor for time-lapse imaging of apoptotic processes and degeneration. [Nat Methods 7\(1\): 67–73.](#)
2. Kim, Y.E. *et al.* (2010) (b) Monitoring apoptosis and neuronal degeneration by real-time detection of phosphatidylserine externalization using a polarity-sensitive indicator of viability and apoptosis. [Nat Protoc. 5\(8\): 1396-405.](#)

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**Storage** Store at +4°C. DO NOT FREEZE.  
This product should be stored undiluted. This product is photosensitive and should be protected from light.

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**Guarantee** 6 months from date of despatch.

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**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 \(ANNEX200APC\)](#)

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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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