Datasheet: APO004

BATCH NUMBER 154355

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

	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^{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.

Reagents In The Kit

pSIVA-IANBD 200 µl
Propidium Iodide Staining Solution 500 µl

Instructions For Use

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.* 2010b). If Ca^{2+} levels are insufficient, supplement the culture medium with 2 mM Ca^{2+}.

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^{2+}, 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.

Instructions for use can be found at www.bio-rad-antibodies.com/uploads/IFU/APO004.pdf

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.

Storage

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

Guarantee

6 months from date of despatch

Acknowledgements	pSIVA^{TM} is a trademark of Novus Biologicals and is protected under patent no. 8.541.549.
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)
Regulatory	For research purposes only

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

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