ab129817

Kinetic Apoptosis Kit (Microscopy)

Instructions for Use

A polarity sensitive, reversible-binding probe for detecting exposed PS residues in the lipid membrane bilayer.

View kit datasheet: www.abcam.com/ab129817
(use www.abcam.cn/ab129817 for China, or www.abcam.co.jp/ab129817 for Japan)

This product is for research use only and is not intended for diagnostic use.

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1. Overview

Abcam's Kinetic Apoptosis kit is based on pSIVA™(Polarity Sensitive Indicator of Viability & Apoptosis) probe. This is an Annexin XII based, polarity sensitive probe for the spatiotemporal analysis of apoptosis and other forms of cell death (Fig. 1). pSIVA™ binding is reversible which enables researchers, for the first time, to detect irreversible as well as transient phosphatidylserine (PS) exposure (Fig. 2). PS exposure is a hallmark phenomenon occurring early during apoptosis and persisting throughout the cell death process and is most often considered to be irreversible. However, transient PS exposure is increasingly being recognized as a phenomenon in its own right and described to occur during both normal physiological processes and reversible or rescuable apoptotic/cell death events (*Kim et al 2010a/b*).

2. Background

pSIVA™ is conjugated to IANBD, a polarity sensitive dye that fluoresces only when pSIVA™ is bound to the cell membrane (Fig. 3). pSIVA-IANBD fluorescence is measured using conventional FITC filter sets. The unique properties of pSIVA™ allow researchers to gain additional information on cell death/cell survival processes compared to Annexin V conjugates, which fluoresce irrespective of whether or not they are bound to PS. pSIVA-IANBD applications

include flow cytometry (ab129816) and live cell fluorescence microscopy imaging (ab129817).

pSIVA™-IANBD is polar sensitive. pSIVA™-IANBD fluoresces in non-polar but not polar environments. When pSIVA™-IANBD is bound to PS it is in the non-polar environment of the membrane lipid bilayer and fluoresces. However, when pSIVA™-IANBD is not bound it is in the polar environment of the media or buffer and does not fluoresce.

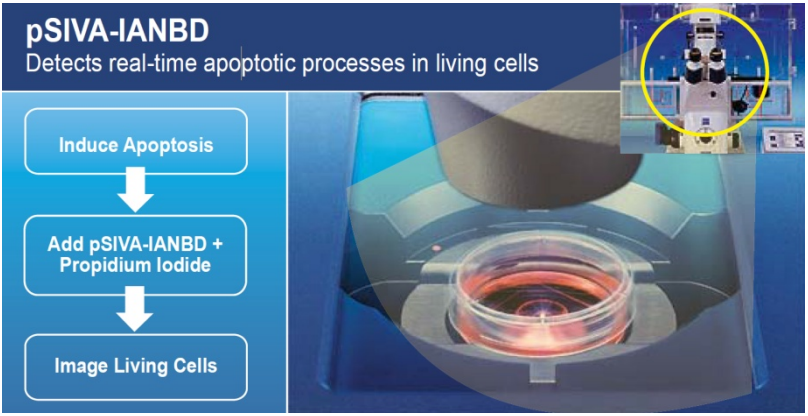

Figure 1. Monitoring apoptosis real-time in living cells with pSIVA-IANBD. pSIVA-IANBD binds to cells undergoing apoptosis and can be present in the culture media during the duration of the experiment since it only fluoresces when actually bound to cells. This feature enables time lapsed imaging to monitor the progression of apoptosis and other phenomenon where pSIVA-IANBD binds to cells. pSIVA-IANBD can also be used for flow cytometric, *in vivo* and high throughput applications.

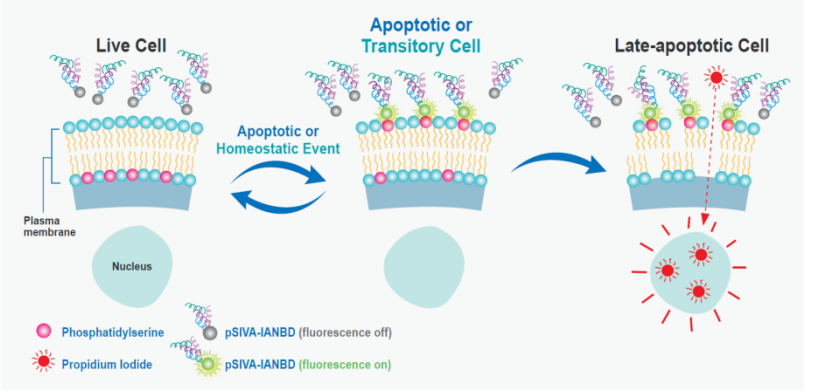

Figure 2. pSIVA-IANBD: A polarity sensitive, reversible-binding probe for detecting exposed PS residues in the lipid membrane bilayer. PS translocation from the cytoplasmic to external face of the membrane occurs early in apoptosis and has been considered to be an irreversible event. However, it is now that known that PS exposure may also be ephemeral or reversible. PS flipping back to the cytoplasmic face following homeostatic or rescuable cell death events results in release of PS-bound pSIVA-IANBD and loss of fluorescence. PI is a vital dye used to identify late apoptosis or necrosis which are both associated with loss of membrane integrity.

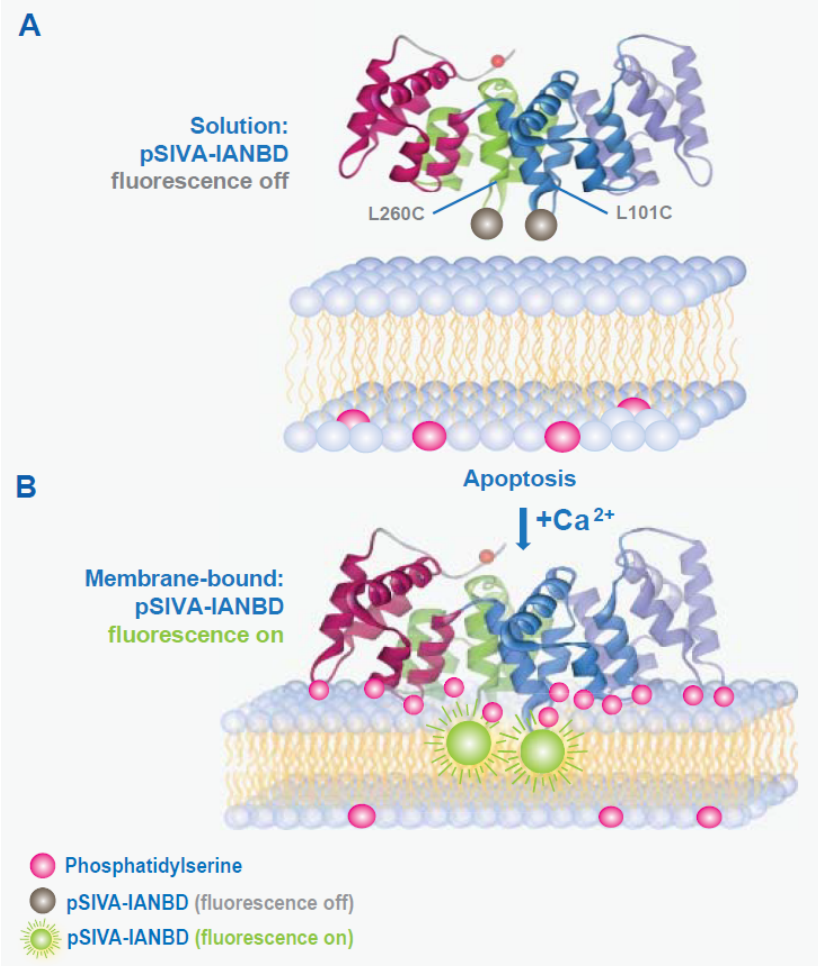

Figure 3. Structure-based design of pSIVA™ depicted with the crystal structure of Annexin B12 along with the introduced L101 and L260 cysteine (C) mutations. The cysteine IANBD fluorophore labels are denoted as grey spheres (non-fluorescing) or green stars (fluorescing).

A. PS is primarily localized on the inner leaflet of the plasma membrane of healthy cells, and therefore inaccessible to pSIVA-IANBD. Hence, pSIVA-IANBD remains in solution and the polarity-sensitive IANBD fluorophores do not emit any significant fluorescence (grey spheres).
B. Early during apoptosis, PS translocates to the outer leaflet and becomes accessible to pSIVA. When pSIVA-IANBD bind to PS, the IANBD labels are exposed to the nonpolar lipid environment of the membrane which results in a 'switching on' of the IANBD fluorescence signal. Adapted from *Kim et al 2010b*.

Features and advantages

- Advanced PS Exposure Probe
- More applications than Annexin V-conjugates (Table I)
    - Polarity sensitive fluorescence
    - Fluoresces when bound to PS
    - PS binding is reversible
    - Detects both transient and irreversible PS exposure

Features	pSIVA-IANBD	Annexin V-FITC
Non-toxic	✓	✓
Detect PS exposure	✓	✓
Detect early apoptosis	✓	✓
Flow cytometry	✓	✓
Viability assessment: Flow cytometry	✓	✓
Toxicity assays	✓	✓
Fluoresces only when bound to PS	✓	
No washing required	✓	
Detect transient PS exposure	✓	
Distinguish between transient & irreversible PS exposure	✓	
Live-cell imaging	✓	
Viability assessment: Live-cell imaging	✓	
In vivo imaging	✓	
High-throughput screen	✓	

Table I. Comparison of pSIVA-IANBD and Annexin V-FITC. pSIVA™ is a new generation Annexin for detecting PS exposure, with more features than Annexin V.

Applications

The pSIVA™ assays are extremely straightforward: pSIVA-IANBD or pSIVA-IANBD + Propidium Iodide (PI) is directly added to cells or tissues, incubated and analyzed.

Applications for pSIVA™ include flow cytometry (ab129816) and live cell fluorescence microscopy fluorescence imaging (ab129817). pSIVA™ has been used to analyze PS exposure in various cell lines including Jurkat (Fig 4), HL60 (Fig 5), COS-7 (*Kim et al, 2010a*), HEK293 (*Kim et al, 2010b*) and Neuro2A (*Zhang et al, 2011*) as well as with primary dorsal root ganglion (DRG) neurons (*Kim et al, 2010b*). Since pSIVA, like Annexin V binds to PS, it should be useful for virtually all the applications, cell types and methods described for Annexin V. However, whereas Annexin V has technical limitations in live cell imaging, pSIVA™ has been optimized for this technique (*Kim et al, 2010a/2010b*).

Live-cell imaging is a vital application for studying dynamic biological processes in real time. Synthetic fluorophores and fluorescent proteins are both qualitative and quantitative reporters of intracellular structure and dynamics in live-cell imaging. Heretofore, there has been a lack of available reporters for studying the process of cell death/cell survival in live-cell imaging. Annexin V-conjugates are impractical for live-cell imaging because separate steps are required for binding of the probe and subsequent removal of the unbound Annexin V-conjugate to reduce the background fluorescence before analysis. In contrast, unbound pSIVA-IANBD does not fluoresce and hence there is no washing step nor unwanted background.

pSIVA's most notable live-imaging applications include monitoring the subcellular onset of PS externalization, tracking PS exposure

throughout the death process, and demonstrating reversible PS exposure events associated with rescuable cell death (*Kim et al, 2010a/2010b*). The advent of pSIVA, a novel PS exposure reporter, for live-cell imaging of cell death/cell survival processes may very well be the most significant assay advance in the Cell Death field today.

3. Protocol Summary

Induce Apoptosis in Sample Cells

⇩

Add 10 -20 μl /ml pSIVA-IANBD + 5-10 μl/ml PI directly to cultures.

Omit PI for one color pSIVA-IANBD staining

⇩

Observe under the microscope.

⇩

Results may be seen immediately or within 5-10 min.

4. Storage and Handling

A. Kit Components

Component	Amount	Storage conditions	Notes
pSIVA-IANBD	200 µl	2-8 °C	10-20 µl/ml
Propidium Iodide Staining Solution	500 µl	2-8 °C	5-10 µl/ml

B. Additional Materials Required

The following are key additional items needed or recommended; they are not included.

- Live-imaging setup (see *Kim et al, 2010a/b* for additional information)
- Experimental cells
- Positive control cell line (recommended, not required). Researchers may want to include a cell line such as Jurkat which is easily induced to undergo apoptosis as a positive control.
- 2 mM Ca^{2+} solution (if the culture medium lacks Ca^{2+})

5. Protocol

The Kinetic Apoptosis assay is based on pSIVA, a novel Annexin B12 probe, conjugated to IANBD, a polar sensitive dye to detect the

cell surface exposure of PS. pSIVA™ is a Ca^{2+} dependent binding protein that has high affinity and selectivity for PS, and pSIVA-IANBD fluoresces only when bound to PS (Figs. 2,3). PS on the cell surface is a generally accepted hallmark of cells in apoptosis and is one of the most widely used markers to detect apoptosis.

PS is kept localized to the inner leaflet of the plasma membrane by an ATP-dependent aminophospholipid translocase in healthy cells that flips PS from the outer to inner membrane. However, PS accumulates by diffusion in the outer leaflet if energy in the form of ATP becomes unavailable or if the plasma membrane loses integrity. In cells undergoing apoptosis, the PS that is normally located on the inner leaflet is translocated to the outer leaflet where it is exposed to the external milieu. Exposure occurs in the early phases of apoptosis while the membrane is still intact, before plasma membrane integrity is lost. PS exposure may parallel caspase-3 activation, another early marker of apoptosis.

PS exposure, however, is not unique to apoptosis but also occurs during other forms of cell death such as necrosis and even during normal physiological events. Both late stage apoptosis and necrosis are associated with leaky or permeable cell membranes. Hence, pSIVA-IANBD assays can be performed in conjunction with a vital dye such as propidium iodide (PI) to distinguish between non-apoptotic (pSIVA-IANBD negative/PI negative), early apoptotic

(pSIVA-IANBD positive/PI negative), and late apoptotic or necrotic cells (pSIVA-IANBD positive/PI positive) (Table II).

	pSIVA-IANBD	PI
Healthy cells	-	-
Transient PS Exposure	+	-
Reversal of PS Exposure	-	-
Early Apoptosis	+	-
Late Apoptosis	+	+
Necrosis	-/+	+

Table II. Tracking Cell Death and PS Exposure Events with pSIVA-IANBD and PI. Should PS flip back from the outer to inner membrane, pSIVA-IANBD-PS binding will be lost and pSIVA-IANBD will be released in the media and no longer fluoresce. Necrosis may happen so quickly that PS binding sites are destroyed or inaccessible due to rapid loss of membrane integrity.

The concept that PS exposure is an irreversible event in the apoptotic program has been a long standing dogma in the cell death field. pSIVA-IANBD assays have demonstrated that reversal of PS exposure can parallel rescue of cells from imminent death (*Kim et al, 2010a*). During rescue pSIVA-IANBD is released from the cell membrane into the external milieu, and PS-bound dependent fluorescence is lost (Table II). PS-bound dependent fluorescence and reversible binding are among the most salient features of pSIVA-IANBD over classical Annexin V-conjugate assays. PS-bound dependent fluorescence can be particularly advantageous in

microscopy assays since unbound pSIVA-IANBD lacks fluorescence, and therefore background signal. This is in contrast to Annexin V-fluorescent conjugates which fluoresce irrespective of whether or not they are bound to PS, resulting in background signal and a loss of sensitivity.

As such, pSIVA-IANBD measures exposed PS, and when used in conjunction with PI can track the loss of membrane integrity occurring during the process of cell death. It is important to note that the pSIVA™ assay, like Annexin V assay, identifies exposed PS and loss of membrane integrity irrespective of the particular term used to describe cell death. Researchers are encouraged to consult the scientific literature for additional information about PS exposure and loss of membrane integrity during the process of various types of cell death.

Protocols

Before you begin

Please note that pSIVA-IANBD binding to PS requires the presence of Ca^{2+} , and that sufficient levels are usually present in most cell culture medium formulations (~1-2 mM Ca^{2+}) (*Kim et al, 2010B*). However if your medium lacks Ca^{2+}, it should be supplemented with 2 mM Ca^{2+} prior to adding pSIVA-IANBD.

Reagents

1. pSIVA-IANBD: Use 10-20 μl/ml.
2. PI: Use 5-10 μl/ml. Note: PI, a ready-to-use nucleic acid dye, is used for two-color (pSIVA-IANBD + PI) assays. Omit PI for one color pSIVA-IANBD assays.

Basic Fluorescence Microscopy

Staining

1. Add 10-20 μl /ml pSIVA-IANBD + 5-10μl/ml PI directly to cultures. Omit PI for one color pSIVA-IANBD staining
2. Observe under the microscope. Use the green fluorescence filter set for pSIVA-IANBD (excitation maximum 488 nm and emission maximum 530 nm) and a red fluorescence filter set for PI (omit PI for one color staining).

3. Results may be seen immediately or within 5-10 min. Since apoptosis is a dynamic process, results may change over time.

Note: pSIVA-IANBD and PI dilutions are provided as guidelines, researchers may need to optimize for their own model systems.

Time-Lapse Live Cell Imaging: Introductory Remarks

pSIVA-IANBD is added directly to cell cultures to image real-time, chronological and dynamic events occurring during apoptosis (Fig 4):

1. Monitor progression of cell death.
2. Monitor cell-to-cell variations in response to treatments.
3. Assess differences in vulnerabilities of individual cells to death stimuli.
4. Identify where the first subcellular responses occur can be monitored.
5. Measure viability of cells without perturbing experimental conditions.

A gradual increase in pSIVA-IANBD staining was seen in treated cells which was concurrent with progression towards cell death as indicated by PI staining during later time points. Little or no staining was seen in untreated cells.

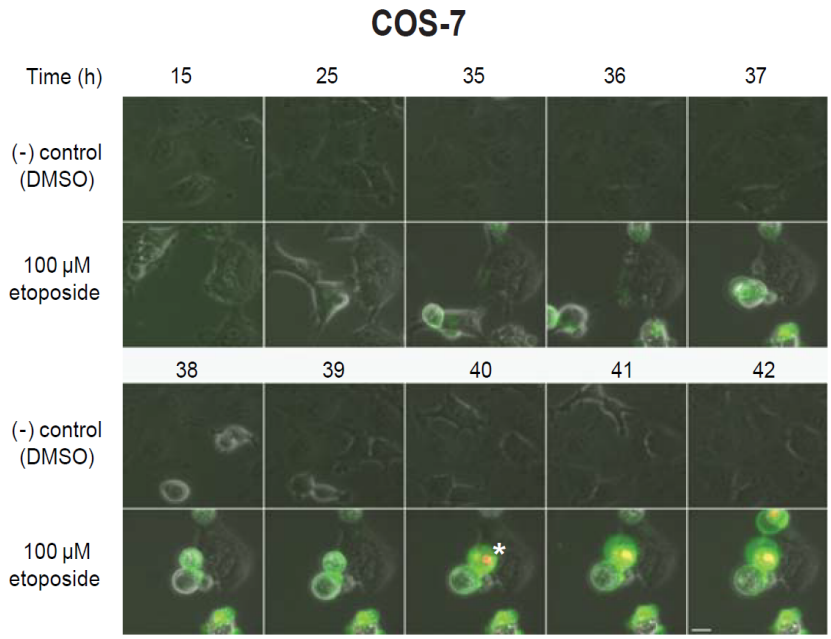

Figure 4. Real-time live cell imaging of apoptosis: COS-7 cells. COS-7 cells were induced to undergo apoptosis with etoposide (100 µM) or left untreated [DMSO (-) control]. pSIVA-IANBD + PI was added directly to the culture media and cells were imaged (37°C, 5% CO_2) by time lapse microscopy of the same fields over time. Merged channels of green and red fluorescence are shown. Green fluorescence indicates pSIVA-IANBD binding to PS exposed on the outer leaflet of the plasma membrane, and orange-yellow fluorescence indicates PI staining of nuclei. pSIVA-IANBD stained cells prior to PI staining. *Time point when PI staining was first seen in the cell; PI staining indicates loss of membrane integrity which is characteristic of late apoptotic or dead cells. Figure from *Kim et al (2010a)*.

Time-lapse Imaging: Basic protocol

This protocol is adapted from *Kim et al (2010b)*. The optimal protocol for your experiments may vary depending on your imaging equipment and experimental model system. A six-well plate is used as an example, other configurations may be also be used.

1. Turn on the fluorescence lamp, incubation system (37°C, 5% CO_2 in a humidified atmosphere), motorized stage, microscope and camera. Let the system equilibrate at 37°C for 2-3 h. Also equilibrate the plate temperature in the microscopy incubation chamber ~ 1h before imaging. Equilibration is an essential step for time-lapse imaging experiments using an automated system with programmed positions and focus points because slight changes in temperature in the system can disrupt the focus.
2. Add 10-20 μl/ml pSIVA-IANBD and 5-10 μl/ml to cultures. Use one well as a negative control (no reagents).
3. Set up the imaging program with the microscope software as follows: time course of imaging and time interval for capturing images.
4. Set fluorescence channels and exposure times. For pSIVA™ use the green fluorescence filter set (excitation maximum 488 nm and emission maximum 530 nm). Minimize bleaching during extended time-lapse imaging experiments by decreasing the exposure time and/or incorporating an EMCCD (electron multiplying charged coupled device) camera for the time-lapse

experiments (for example, Photometrics Cascade 1K). Note: Particular care should be taken for experiments aimed at imaging the reversal of pSIVA-IABND binding, which is indicative of transient PS exposure, rescue from apoptosis/cell death or rescue from neuronal degeneration.

5. Pick the positions and focal depth for the different fields of view to be imaged.
6. Recheck the position and focus for each field of view before starting the time-lapse imaging experiment.
7. Analyze results at the end of experiment.

Detection of Transient PS Exposure

Although PS exposure is most well known as an irreversible event that occurs during apoptosis, the phenomenon of transient exposure in healthy cells is increasingly being recognized (Fig. 5). For example, transient PS exposure occurs during lymphocyte activation, vesicle release, muscle cell differentiation, embryonic development and other events that are accompanied by changes in plasma membrane structure (*Kim et al, 2010b*).

Methodology: Transient PS exposure has been studied by live imaging. Transient versus apoptotic PS events can be distinguished by setting the time frame of imaging to match the time frame of the process (*Kim et al, 2010b*). For example, transient PS exposure during vesicle release in healthy cells may occur on a time scale of seconds to minutes before PS is restored to the inner leaflet. In

contrast, PS remains exposed on the outer leaflet for hours to days during apoptosis, either until cell death or rescue occurs. As the phenomenon of transient PS exposure is an emerging area of study, researchers will need to empirically determine the optimal imaging time frames for their model system and the process they are evaluating.

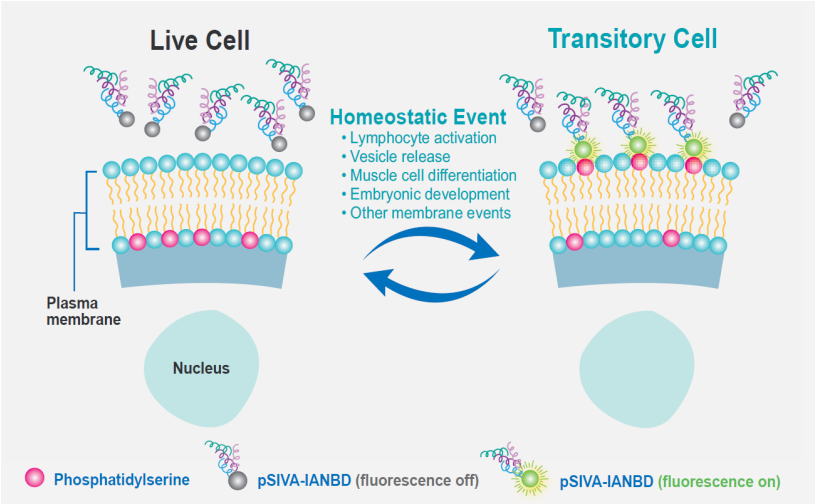

Figure 5. Ephemeral or fleeting pSIVA-IANBD fluorescence signals accompany transient PS exposures associated with normal physiological or homeostatic events (artist's rendition). When PS flips back to the inner membrane following transient PS exposure, pSIVA-IANBD will be released back into the medium and fluorescence lost. Potential areas of study are shown in the cartoon. Note: The phenomenon of transient pSIVA-IANBD fluorescence exposure has been observed in unperturbed cultures as rapid on/off fluorescence, and is thought to represent normal membrane event. However, this is a wide open area of study and details remain to be elucidated.

Protocol examples for transient PS exposure

Analysis of Degeneration and Rescue: Primary Neurons

DRG neurons are useful model systems for survival and degeneration because they are only dependent on one growth factor for survival, NGF, which can easily be removed. Removal of NGF leads to degeneration. Axonal degeneration involves apoptotic mechanisms which do not always result in cell death. Hence, neuronal degeneration/rescue studies can be used to help define critical periods or windows when apoptosis processes are still reversible (reviewed in *Kim et al, 2010a/b*).

The protocols are adapted from *Kim et al, 2010a/b*. The experiments were done with six-well plates* of purified primary dorsal root ganglion (DRG) that were still dependent on NGF for survival (cultured 1-2 weeks). The DRG neurons were cultured in etched wells in order to orient growth along a single axis. Note: For NGF deprivation experiments, neurons should be used after 1-2 weeks because after about 3 weeks the neurons will be independent of NGF for survival.*Experiments should ideally be performed on tissue culture treated plastic. If using glass coverslips, they must be attached to culture wells before plating cells to prevent coverslips from shifting during imaging. Coverslips can be attached to wells with nontoxic adhesives (silicon caulk, cyanoacrylate) or with a drying substrate such as collagen.

Neuronal Degeneration Protocol

1. Begin with Step 1 of the “Time-lapse imaging: Basic protocol” to equilibrate microscopy system.
2. Remove NGF from the culture media to initiate degeneration in specific wells. Wash cells three times with medium without NGF. Other suitable protocols may also be used to induce degeneration. For a negative control (no induction of degeneration), replace the NGF back into one well.
3. Follow Steps 2-7 of the “Time-lapse imaging: Basic protocol. Note: An imaging interval of 30 m is suggested, neurons will degenerate over 24-40 h.

Anticipated results: PS exposure on the axon can be detected by pSIVA-IANBD as early as 10 h in 1 week old DRG neurons that have been induced to undergo degeneration by removal of NGF (*Kim et al, 2010b*). PS exposure has been found to start first in localized areas of the axon and then spread progressively in either direction, towards the cell body or axon (Figs. 6 and 7). pSIVA-IANBD and PI staining in the cell body occurred later (Fig. 8). There will likely be cell-cell variability and heterogeneity in the initiation and progression of PS exposure, and thus pSIVA-IANBD fluorescence patterns in the degenerating neurons.

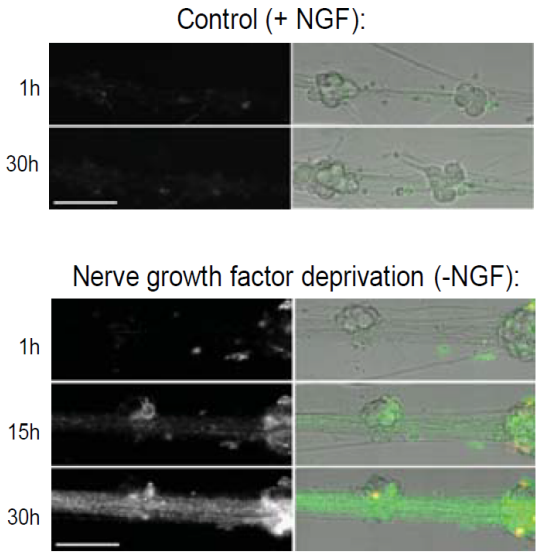

Figure 6. Monitoring the degeneration of neurons with pSIVA-IANBD. Time-lapse microscopy of rat DRG neurons with NGF (normal physiological conditions) (a) and under NGF deprivation (b). pSIVA-IANBD + PI were present in the culture medium for the duration of the experiment. pSIVA-IANBD fluorescence (left panels) and merged images of phase contrast, green (pSIVA-IANBD) and red (PI) fluorescence (right panels) are shown. Increasing fluorescence was seen -NGF but not +NGF cultures over time, indicating neuronal degeneration in the -NGF but not +NGF cultures. 100 um scale bar. Figure from *Kim et al (2010a)*.

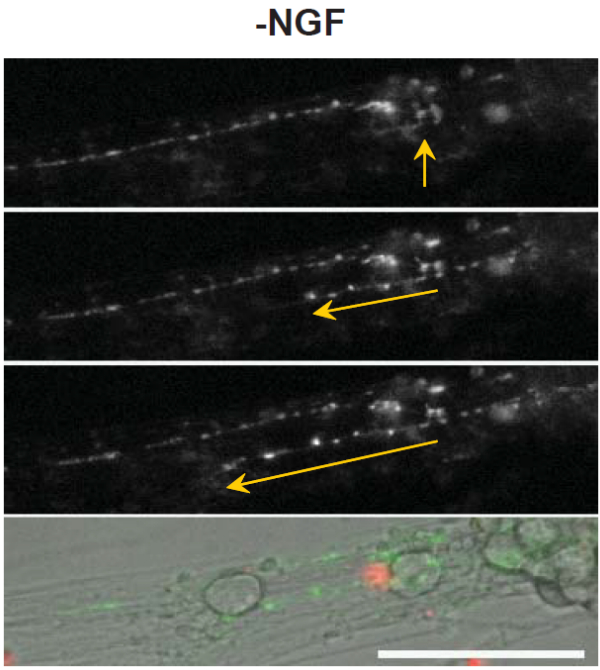

Figure 7. Spatiotemporal analysis of membrane changes along a single degenerating axon with pSIVA-IANBD. Time-lapse images at 20 min intervals of a rat DRG axon from a -NGF culture showing progression of the pSIVA-IANBD punctuate staining of PS exposure. Black and white: pSIVA-IANBD fluorescence. Bottom panel: and merged images of phase contrast, green (pSIVA-IANBD) and red (PI) fluorescence. 100 um scale bar. Cells were imaged with pSIVA-IANBD and PI present in the culture medium for the duration of the experiment. Figure from *Kim et al (2010a)*.

Neuronal Rescue Protocol

1. Neuronal degeneration is initiated as described in Step 2 of the Neuronal Degeneration Protocol.

2. Add NGF back to cultures once PS exposure (pSIVA-IANBD fluorescence) is detected on the axons. Different time points should be used to determine the window of rescuability in your model system. For example, *Kim et al (2010a)* added NGF back to cultures at 7, 10 and 15 h after initial NGF removal (Fig. 9).

3. Quantify the recovery from degeneration by measuring the total fluorescence of different fields of view at low magnification in both control (-NGF) and rescued (-NGF followed by +NGF) cultures. Increasing pSIVA-IANBD fluorescence indicates increasing PS exposure. Decreasing fluorescence in rescued cultures is considered to indicate reversal of PS exposure.

Expected results: Adding NGF back to the cultures will result in a reduction of fluorescence in neurons that have been rescued (Figs. 9 and 10). Neurons already showing signs of apoptosis (pSIVA-IANBD) will vary in their ability to be rescued. There appears to be a critical window for rescuability with respect to pSIVA-IANBD fluorescence (*Kim et al 2010a/b*). pSIVA-IANBD positive staining typically begins in the axon and moves towards the cell body. Cells appear to be losing their ability to be rescued once pSIVA-IANBD staining is seen in the cell body (Fig. 11).

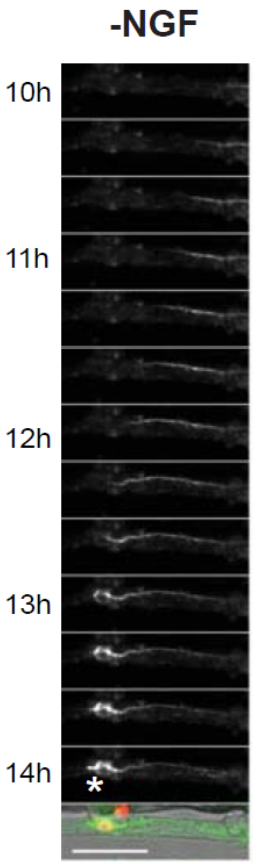

Figure 8. Time-lapse images showing the progressive movement of PS exposure along axons to the cell body as detected by pSIVA-IANBD. Images were taken 10-14 h (3 frames/h) after NGF removal. *Indicates where PI staining was first seen in the cell body. Black and white: pSIVA-IANBD fluorescence. Bottom panel: Merged images of phase contrast, green (pSIVA-IANBD) and red (PI) fluorescence. 100 μm scale bar. Cells were imaged with pSIVA-IANBD and PI present in the culture medium for the duration of the experiment. Figure from *Kim et al 2010a*.

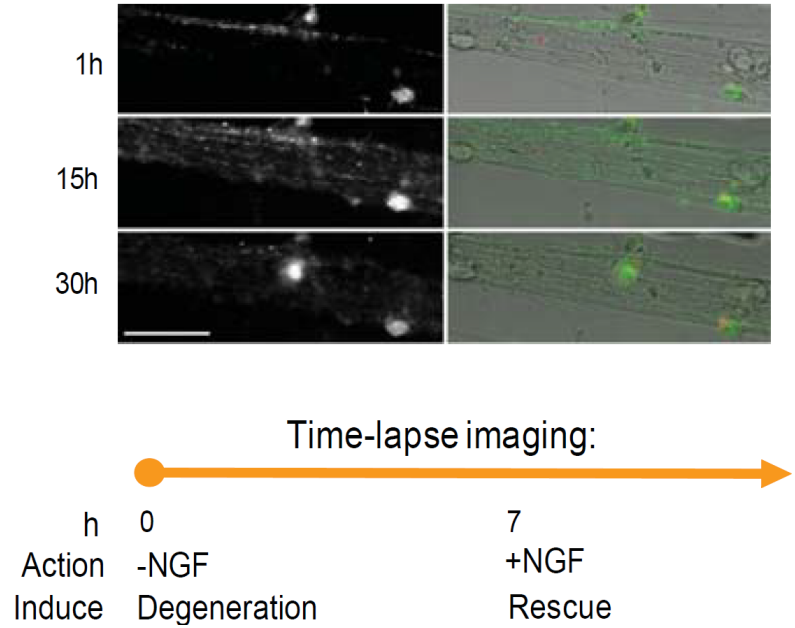

Figure 9. Rescue of axonal degeneration. DRG neurons that were dependent on NGF were induced to undergo cell death by NGF deprivation for 7 h before re-addition of NGF to the culture media. Note the reduction in fluorescence between 15 h and 30 h, indicative of a reduction of pSIVA-IANBD binding. See Fig. 12 for a quantitative analysis of rescued versus non rescued cultures. Black and white: pSIVA-IANBD fluorescence. Color: Merged images of phase contrast, green (pSIVA-IANBD) and red (PI) fluorescence. 100 um scale bar. Cells were imaged with pSIVA-IANBD and PI present in the culture medium for the duration of the experiment. Figure from *Kim et al 2010a*.

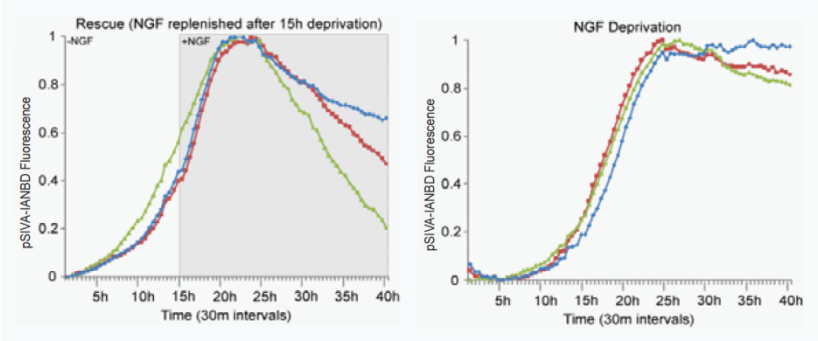

Figure 10. Quantitative analysis of pSIVA-IANBD fluorescence in rescued and NGF deprived neuronal cultures. Rescue of axonal degeneration. DRG neurons that were dependent on NGF were induced to undergo cell death by NGF deprivation 15 h before re-addition of NGF to the culture media. Total fluorescence was measured for different fields at low magnification. pSIVA-IANBD fluorescence decreased in cultures where NGF was re-added compared to cultures which were deprived of NGF throughout the entire 40 h monitoring period. Variations in the amount of rescue (decreasing total fluorescence) reflects heterogeneity in the ability of individual neurons to be rescued as well as differences in the time frame needed for rescue. Cells were imaged with pSIVA-IANBD and PI present in the culture medium for the duration of the experiment. Figure from *Kim et al 2010a*.

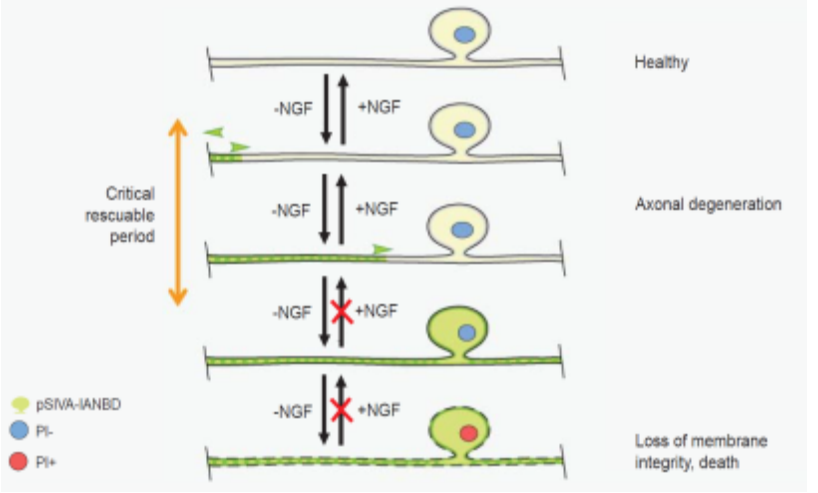

Figure 11. Critical window for neuronal cell rescue. pSIVA-IANBD staining indicates that PS exposure during apoptosis starts on the axons and initially may move towards or away from the cell body. Eventually the cell body becomes positive, followed by PI staining indicating loss of membrane integrity and impending death. Rescue appears to be possible only before PS exposure/pSIVA-IANBD fluorescence has progressed to the cell body. Green: pSIVA-IANBD fluorescence. Red: PI fluorescence.

6. pSIVA™ General References

1. Kim YE, J Chen, JR Chan, R Langen. Engineering a polarity-sensitive biosensor for time-lapse imaging of apoptotic processes and degeneration. *Nat Methods 7:67-73 (2010a).* **Real-time live-cell imaging and time-lapse microscopy of apoptosis: Fig 2 (COS-7 cells), Fig 3 (rat neuronal degeneration), Fig 4 (rat axonal degeneration), Fig 5 (rescue of rat neuronal degeneration as visualized by pSIVA).**

2. Kim YE, J Chen, R Langen, JR Chan. 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 (2010b).* **Time lapse microscopy of neurons in normal survival conditions and after NGF deprivation (Fig 2).**

3. Zhang CQ, Yeh T-l, A Leyva, LG Frank, J Miller, YE Kim, R Langen, S Finkbeiner, ML Amzel, CA Ross, MA Poirier. A compact B model of huntingtin toxicity. *JBC 286:8188-8196 (2011).* **A pSIVA-IANBD based cell suspension toxicity assay was used to determine cell viability in mouse Neuro2A (neuroblastoma) overexpressing huntingtin proteins (Fig 4).**

7. FAQ & Troubleshooting

Frequently asked questions

1. **Is pSIVA-IANBD in the cell culture harmful to cells?** We do not have any evidence that pSIVA-IANBD is harmful to cells or perturbs the cell culture. Furthermore, no differences in cell growth rate were observed when COS-7 cells were incubated in the presence or absence of pSIVA-IANBD (*Kim et al, 2010a*).
2. **Does unbound pSIVA-IANBD in solution have background fluorescence?** pSIVA-IANBD fluorescence in the solution (unbound) state is negligible in comparison with the membrane-bound state (~50 fold increase) (*Kim et al, 2010B*).
3. **If pSIVA-IANBD is inadvertently used in excess, will background increase?** Since unbound pSIVA-IANBD lacks fluorescence, background will not appreciably increase in the presence of moderate excess of pSIVA-IANBD.
4. **Can pSIVA-IANBD be used for high-throughput screening?** Yes since pSIVA-IANBD fluorescence correlates directly with binding to apoptotic cell membranes, pSIVA-IANBD fluorescence can be quantified using a multiplate reader for high-throughput screening of conditions that cause or prevent cell death.

Troubleshooting Guide

Problem	Reason	Solution
No pSIVA-IANBD fluorescence	Ca^{2+} is not present in the culture media	Check medium formulations and make sure that the Ca^{2+} ($CaCl_2$) concentration is ~1-2 mM
	Suboptimal microscopy settings	Make sure that the live imaging setup has been equilibrated at 37°C, that the microscope settings are correct for the camera, fluorescence and exposure times, and that the focus positions are correct.
	PS exposure is not present	Use a positive control cell line that is readily induced to undergo apoptosis to validate the system

For further technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select "*contact us*" on www.abcam.com for the phone number for your region).

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