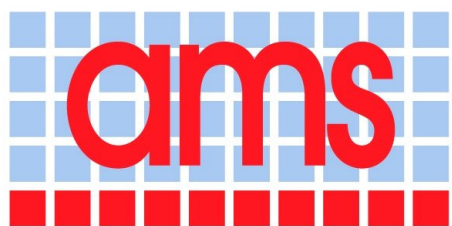
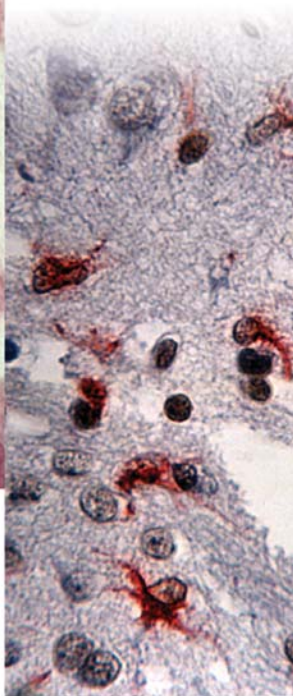
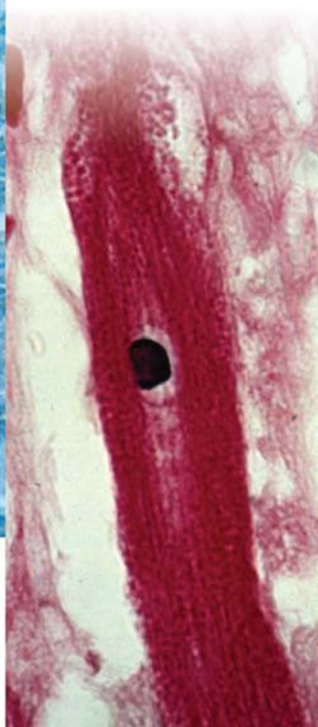
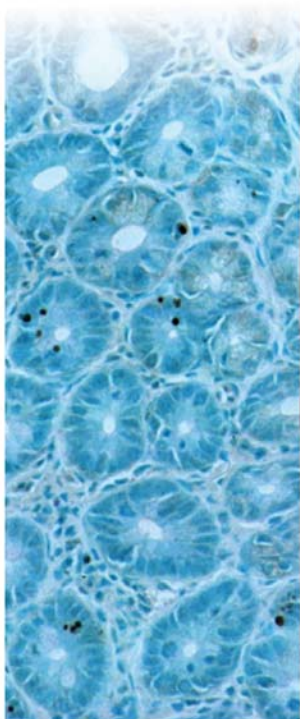


*In Situ*  
**APOPTOSIS  
DETECTION  
KITS**



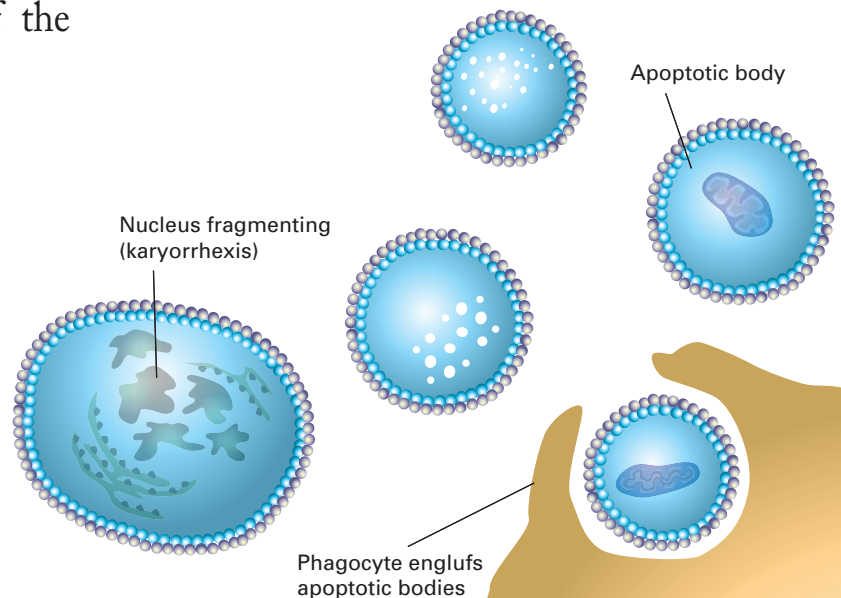
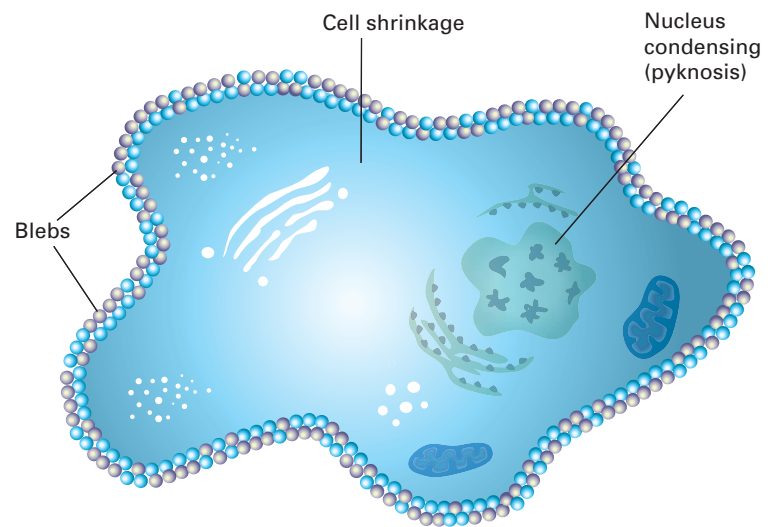
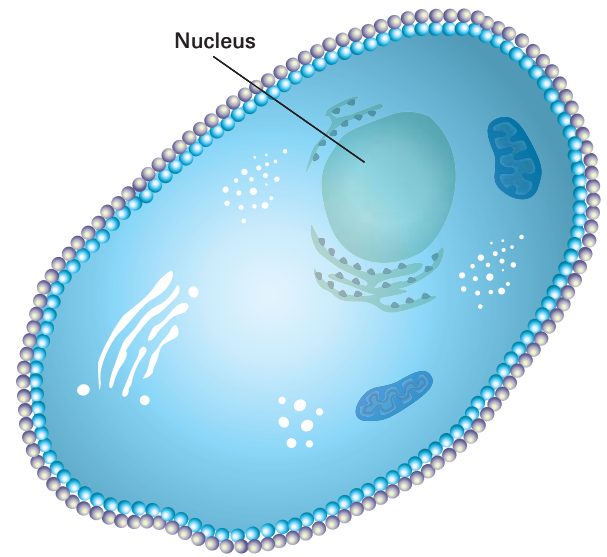
biotechnology (europe) ltd

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# APOPTOSIS

Apoptosis or programmed cell death is a mechanism of cellular suicide that occurs after sufficient cellular damage. Apoptosis results in the condensation of the nucleus, and the cell shrinks. Chromosomal fragmentation due to the controlled digestion of DNA is visible in apoptotic cells. Cytoplasmic blebbing and apoptotic bodies are also seen during apoptosis. The end result of apoptosis is cell death without inflammation of the surrounding tissue.



# PRODUCTS

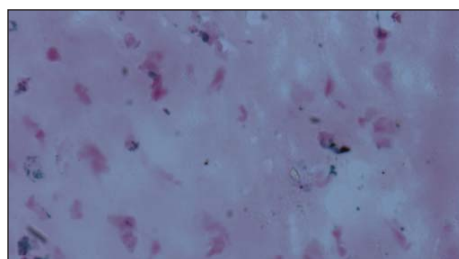
## *Tissue Specific TACS™ Kits for In Situ Apoptosis Detection*

Trevigen's Tissue Specific TACS™ Kits were developed to provide researchers with an effective method for identifying apoptotic cells in specific tissue types. These complete kits provide all the reagents required for labeling including permeabilization reagents, labeling and stop buffers, labeling and detection reagents, TACS-Nuclease™ reagent, and cations. The kits also include detailed protocols with hints and tips for optimal labeling of samples.

Description	Specificity	Size	Catalog No.
CardioTACS™ in situ Apoptosis Detection Kit	Cardiac Tissue	30 Samples	4827-30-K
DermaTACS™ Kit in situ Apoptosis Detection Kit	Dermal Tissue	30 Samples	4829-30-K
NeuroTACS™ II in situ Apoptosis Detection Kit	Neural Tissue	30 Samples	4823-30-K
TumorTACS™ Kit in situ Apoptosis Detection Kit	Tumor Tissue	30 Samples	4815-30-K
VasoTACS™ Kit in situ Apoptosis Detection Kit	Vascular Tissue	30 Samples	4826-30-K

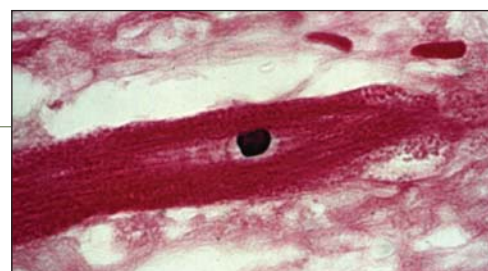
### CardioTACS™ Kit

The CardioTACS™ Kit is optimized for identification of apoptotic cells in cardiac samples. Because the high cellularity of cardiac tissue presents problems in permeabilization, the kit comes with two permeabilization reagents to provide options. The kit is based on DNA end-labeling using terminal deoxynucleotidyl transferase (TdT) and a modified nucleotide that is subsequently detected using our TACS Blue Label™ Detection system.



*Ischemic peri-infarct myocardium in an established model of ischemic cardiomyopathy, 6 weeks following coronary ligation. The tissue was treated with the Trevigen CardioTACS™ kit. Nuclei are labeled in red and the TUNEL labeled nuclei stain black.*

*Photo courtesy of Dr. Y. Joseph Woo, M.D., Division of Cardiovascular Surgery, Department of Surgery, University of Pennsylvania School of Medicine, Philadelphia, PA.*



*Apoptotic rat cardiac myocyte labeled using the CardioTACS™ kit. Rat heart tissue was fixed in 4% paraformaldehyde overnight followed by paraffin embedding. Five micron sections were prepared and placed onto glass microscope slides. The samples were processed following the CardioTACS™ kit protocol. Photo courtesy of Dr. J. Zhang, FDA.*

### CardioTACS™ Citation

**Preemptive heme oxygenase-1 gene delivery reveals reduced mortality and preservation of left ventricular function 1 yr after acute myocardial infarction**

Xiaoli Liu, Jeremy A. Simpson, Keith R. Brunt, Christopher A. Ward, Sean R. R. Hall, Robert T. Kinobe, Valerie Barrette, M. Yat Tse, Stephen C. Pang, Alok S. Pachori, Victor J. Dzau, Kofo O. Ogunyankin, and Luis G. Melo  
**Am J Physiol Heart Circ Physiol**, Jul 2007; 293: H48 - H59.

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# PRODUCTS

## DermaTACS™ Kit

The DermaTACS™ Kit is optimized for measuring apoptosis in skin samples. The kit is based on DNA end-labeling with TdT and modified nucleotides. Detection of incorporated molecules is achieved using a chromogenic substrate with a horseradish peroxidase detection system. The extracellular scaffolding in skin tissue can make it problematic to permeabilize and high background is a common problem. The DermaTACS™ Kit includes two permeabilization reagent options for permeabilization along with a detailed protocol with tips for optimal labeling of skin samples. If you wish to use a prepared positive control you may use either the Cell Culture Control Slides (Cat# 4800-30-20) or the Tissue Control Slides (Cat# 4800-30-40). These controls allow you to run through the procedure to become familiar with handling the samples, etc. Each set of Control Slides is shipped with a product information sheet that provides information on the recommended permeabilization method, incubation times, and interpretation of data.



*Detection of DNA fragmentation in UVB irradiated human skin model, EpiDerm™ with DermaTACS™. Samples were provided courtesy of Dr. Patrick Hayden, MarTek Corporation, Ashland, MA.*

## DermaTACS™ Citation

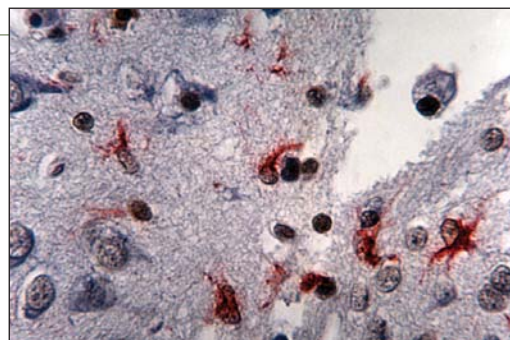
### Use of Topical Insulin to Normalize Corneal Epithelial Healing in Diabetes Mellitus

Ian S. Zagon, Matthew S. Klocek, Joseph W. Sassani, and Patricia J. McLaughlin  
*Arch Ophthalmol*, Aug 2007; 125: 1082 - 1088.

## NeuroTACS™ II Kit

The NeuroTACS™ II Kit is optimized to provide rapid and convenient identification of apoptosis in brain tissue or neuronal cells. The kit has been developed to overcome the common difficulties unique to neuronal samples, including the fragile nature of brain tissue sections, high background problems, poor counterstaining with common dyes, and the need to perform dual labeling experiments to detect cell specific antigens in conjunction with apoptotic cells.

*Double labeling of mouse brain section for apoptosis using NeuroTACS™ II (brown) and a monoclonal antibody to GFAP (red) (not included in kit). Brain sections were fixed in 10% neutral buffered formalin, embedded in paraffin and sectioned at 5 microns. The section was counterstained using Trevigen's Blue Counterstain.*



## NeuroTACS™ Citation

### Caspase 3-Dependent Cell Death of Neurons Contributes to the Pathogenesis of West Nile Virus Encephalitis

Melanie A. Samuel, John D. Morrey, and Michael S. Diamond  
*J. Virol.*, Mar 2007; 81: 2614 - 2623.

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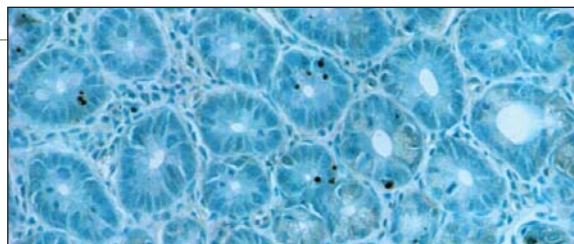
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# PRODUCTS

## TumorTACS™ Kit

The TumorTACS™ Kit provides rapid and convenient identification of apoptosis in tumors or cancer cells. The kit has a unique TUNEL-based system that preferentially labels the double-stranded DNA found in apoptotic cells. The kit contains all the reagents required for permeabilizing, labeling, staining, and counterstaining tumor samples, including TACS-Nuclease™ for preparing positive controls.



*Apoptotic cells within mouse mammary tumor identified using the TumorTACS™ kit. Mammary tumor was fixed in 4% paraformaldehyde overnight followed by paraffin embedding. Five micron sections were prepared and placed onto glass microscope slides. The sample was processed following the TumorTACS™ kit protocol.*

## TumorTACS™ Citation

### **Cathepsin E Prevents Tumor Growth and Metastasis by Catalyzing the Proteolytic Release of Soluble TRAIL from Tumor Cell Surface**

Tomoyo Kawakubo, Kuniaki Okamoto, Jun-ichi Iwata, Masashi Shin, Yoshiko Okamoto, Atsushi Yasukochi, Keiichi I. Nakayama, Tomoko Kadowaki, Takayuki Tsukuba, and Kenji Yamamoto  
**Cancer Res.**, Nov 2007; 67: 10869 - 10878

## VasoTACS™ Kit

The VasoTACS™ Kit is an effective method for identifying apoptotic cells in vascular samples, by reducing background and improving labeling in vascular tissue. Similar to the CardioTACS™ Kit, it is based on DNA end-labeling with TdT and a modified nucleotide detected using our TACS Blue Label™ Detection System.



*Drug induced apoptosis in the small artery of a rat exhibiting spontaneous hypertension using the Trevigen VasoTACS™ Kit. The tissue was formalin-fixed and paraffin-embedded. Photo courtesy of Dr. Jun Zhang, FDA.*

## VasoTACS™ Citation

### **Attenuated herpes simplex virus 1 blocks arterial apoptosis and intimal hyperplasia induced by balloon angioplasty and reduced blood flow**

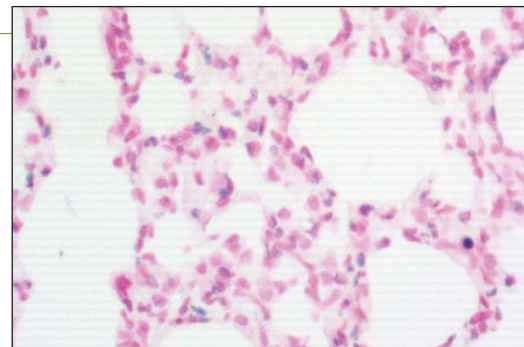
Christopher L. Skelly, Amito Chandiwai, James E. Vosicky, Ralph R. Weichselbaum, and Bernard Roizman  
**PNAS**, Jul 2007; 104: 12474 - 12478.

# PRODUCTS

## All Purpose TACS™ Kits

### TACS•XL® In Situ Apoptosis Detection Kits

TACS•XL® embodies a new approach for the in situ detection of apoptosis. The TACS•XL® kit is based on incorporation of bromodeoxyuridine (BrdU) at the 3' OH ends of the DNA fragments that are formed during apoptosis. The incorporation of BrdUTP by TdT is more efficient than either biotinylated or digoxigenin labeled nucleotides used in other TUNEL-based assays. The detection system utilizes a biotin conjugated anti-BrdU antibody and streptavidin-horseradish peroxidase. The combination of antibody specificity with the signal enhancing properties of biotin:streptavidin results in precise cellular labeling and the highest signal to noise ratio observed in competitive testing. These kits provide all the reagents required for labeling including two permeabilization reagents, labeling and stop buffers, labeling and detection reagents, and TACS-Nuclease™ reagents for generating positive controls with your own samples.



Apoptosis in mouse lung parenchyma tissue detected using TACS•XL® Blue label kit. Photo courtesy of Prof. Francesco D'Agostini, Associate Professor, Department of Health Sciences, Section of Hygiene and Preventive Medicine, University of Genova Via A. Pastore, 1 I-16132 Genova, Italy

### TACS•XL® DAB Citation

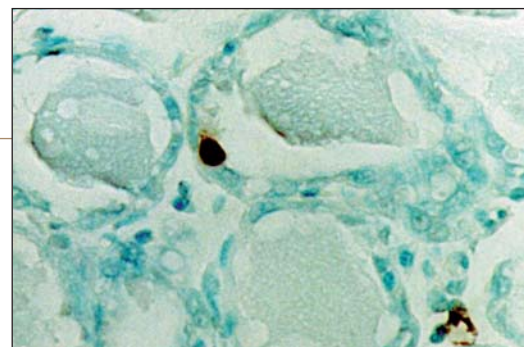
**Osteopontin regulates hindlimb-unloading-induced lymphoid organ atrophy and weight loss by modulating corticosteroid production**

Kathryn X. Wang, Yufang Shi, and David T. Denhardt  
**PNAS**, Sep 2007; 104: 14777 - 14782

Product Description	Stain	Counterstain	Size	Catalog No.
TACS•XL® Blue Label Kit	TACS Blue Label™	Nuclear Fast Red	30 Samples	4828-30-BK
TACS•XL® DAB Kit	DAB	Methyl Green	30 Samples	4828-30-DK
TACS•XL® Replenisher Kit			30 Samples	4828-30-R

### TACS™ 2 TdT In Situ Apoptosis Detection Kits

The TACS™ 2 TdT Kits utilize Trevigen's unique cation optimization system to enhance labeling within particular tissues. A highly purified form of the TdT enzyme is included in the kits for the enzymatic incorporation of biotinylated nucleotides. Biotin labeling is achieved using streptavidin-horseradish peroxidase, and colorimetric substrates diaminobenzidine (DAB) or TACS Blue Label™. For fluorescent detection, a fluorescein conjugate of streptavidin is used and visualized by epifluorescence microscopy. These kits provide all the reagents required for labeling including two permeabilization reagents, labeling and stop buffers, labeling and detection reagents, and TACS-Nuclease™ reagents for generating positive controls with your own samples.



Detection of apoptosis in post-weaning mouse breast tissue using the TACS™ 2 TdT DAB kit. Tissue sections were collected and fixed in 10% neutral buffered formalin, embedded in paraffin and sectioned at 5 µM. Deparaffinized and rehydrated sections were processed following the TACS™ 2 protocol.

### TACS™ 2 TdT Blue Label Citation

**Caspase-3 Gene Deletin Prolongs Survival in Polycystic Kidney Disease**

Yunxia Tao, Iram Zafar, Jun Kim, Robert W. Schrier, and Charles L. Edelstein  
**J. Am. Soc. Nephrol.**, Feb 2008; 10.1681/ASN.2006121378.

[info@amsbio.com](mailto:info@amsbio.com)

Product Description	Label	Size	Catalog No.
TACS™ 2 TdT Replenisher Kit		30 Samples	4810-30-R
TACS™ 2 TdT-DAB Kit	DAB	30 Samples	4810-30-K
TACS™ 2 TdT-Blue Label Kit	TACS Blue Label™	30 Samples	4811-30-K
TACS™ 2 TdT-Fluorescein Kit	Fluorescein	30 Samples	4812-30-K

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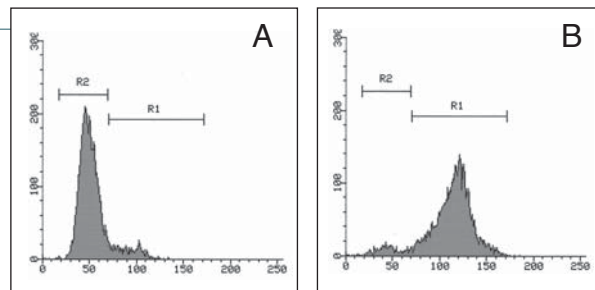
# PRODUCTS

## Specialty TACS™ Kits

Description	Size	Catalog No.
FlowTACS™ in situ Apoptosis Detection Kit	60 Samples	4817-60-K
TiterTACS™ in situ Apoptosis Detection Kit	96 Samples	4822-96-K

### FlowTACS™ Kit

FlowTACS™ takes advantage of Trevigen's exclusive in situ labeling technology to label cell samples for processing by flow cytometry. FlowTACS™ also provides flexibility in selection of fluorophores that are compatible with your research design. The kit is supplied with streptavidin-FITC and allows multi-color labeling in conjunction with experiment specific antibodies. This complete kit provides all the reagents required for labeling including two permeabilization reagents, labeling and stop buffers, labeling and detection reagents, and TACS-Nuclease for generating positive controls with your own samples. The kit can be used for the detection of apoptosis using flow cytometry, direct visualization using a fluorescent microscope or for quantitation using a fluorometer.



Analysis of murine thymocytes at 16 hours after treatment with 10 µg/ml cycloheximide (A) and 1 µM dexamethasone (B). Cells were harvested and labeled according to the FlowTACS™ protocol prior to analysis by flow cytometry. Data courtesy N. Hardegen, NIH, NIDR, Bethesda, MD.

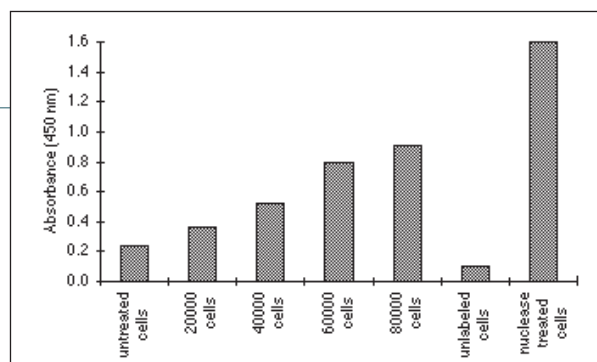
### FlowTACS™ Citation

#### Self-renewal of human embryonic stem cells requires insulin-like growth factor-1 receptor and ERBB2 receptor signaling

Linlin Wang, Thomas C. Schulz, Eric S. Sherrer, Derek S. Dauphin, Soojung Shin, Angelique M. Nelson, Carol B. Ware, Mei Zahn, Chao-Zhong Song, Xiaoji Chen, Sandii N. Brimble, Amanda McLean, Maria J. Galeano, Elizabeth W. Uhl, Kevin A. D'Amour, Johnathon D. Chesnut, Mahendra S. Rao, Anthony Blau, and Allan J. Robins *Blood*, Dec 2007; 110: 4111 - 4119.

### TiterTACS™ Kit

The HT TiterTACS™ Colorimetric Apoptosis Detection Kit takes advantage of Trevigen's exclusive in situ labeling technology bringing it to the 96 well microplate format for high throughput quantitative detection of apoptosis in cultured cells. Detection using TACS-Sapphire™, a non-toxic colorimetric substrate, allows both kinetic and endpoint readings. The reaction generates a blue product that can be measured at 630nm, and will turn yellow after the reaction is stopped with acid allowing endpoint reading at 450nm. The HT TiterTACS™ Kit also provides TACS-Nuclease™ solution to generate positive controls from your own samples giving you a maximal value for the assay.



Detection of apoptosis in ML-1 cells after treatment with 1 µM staurosporine. All control wells contained 1x10<sup>5</sup> cells. Cells were harvested, fixed and labeled according to the TiterTACS™ protocol prior to colorimetric analysis. Reaction was stopped with 2N HCl.

### TiterTACS™ Citation

#### Effects of gamma radiation on FcRI and TLR-mediated mast cell activation

Benjamin P. Soule, Jared M. Brown, Nataliya M. Kushnir-Sukhov, Nicole L. Simone, James B. Mitchell, and Dean D. Metcalfe

*J. Immunol.*, Sep 2007; 179: 3276 - 3286.

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# PRODUCTS

## TACS™ Kit Components

Catalog No.	Component	Size	CardioTACS™ 4827-30-K	DermaTACS™ 4829-30-K	NeuroTACS™ II 4823-30-K	TumorTACS™ 4815-30-K	VasoTACS™ 4826-30-K	TACS XL® Blue Label Kit 4828-30-BK	TACS XL® DAB Kit 4828-30-DK	TACS™ 2 TdT DAB Kit 4810-30-K	TACS™ 2 TdT Blue Label Kit 4811-30-K	TACS™ 2 TdT Fluorescein Kit 4812-30-K	FlowTACS™ 4817-60-K	TiterTACS™ 4822-96-K
4800-30-01	Proteinase K	30 µl	•	•	•	•	•	•		•	•	•		
4800-30-06	Strep-HRP	30 µl	•	•	•	•	•	•	•	•	•		•	•
4800-30-07	DAB Solution	3.75 ml			•	•			•	•	•			
4800-30-11	TACS Blue Label™	3 ml	•	•			•	•		•				
4800-30-12	Blue Strep-HRP Diluent	7.5 ml	•				•			•				•
4800-30-14	Strep Fluorescein	30 µl								•				
4800-30-15	TACS-Nuclease™	15 µl	•	•	•	•	•	•	•	•	•	•	•	•
4800-30-16	TACS-Nuclease™ Buffer	15 µl	•	•	•	•	•	•	•	•	•	•	•	•
4800-30-17	Nuclear Fast Red	50 ml	•					•						
4800-30-19	Red Counterstain C	50 ml		•			•							
4810-30-02	10X TdT Labeling Buffer	100 ml	•	•	•	•	•	•		•	•	•		
4810-30-03	10X TdT Stop Buffer	100 ml	•	•	•	•	•	•	•	•	•	•		
4810-30-04	TdT dNTP	30 µl	•		•	•	•			•	•	•	•	
4810-30-05	TdT Enzyme	30 µl	•	•	•	•	•	•	•	•	•	•	•	
4810-30-09	50X Co <sup>2+</sup>	30 µl								•	•	•		
4810-30-10	50X Mg <sup>2+</sup>	30 µl								•	•	•		
4810-30-14	50X Mn <sup>2+</sup>	30 µl	•		•	•	•			•	•	•	•	
4817-60-02	10X TdT Labeling Buffer	20 ml											•	•
4817-60-03	10X TdT Stop Buffer	20 ml											•	•
4817-60-04	Propidium Iodide/RNase	1 ml											•	
4820-30-01	NeuroPore™	5 ml			•									
4820-30-13	Blue Counterstain	50 ml			•									
4821-96-01	Proteinase K	100 µl												•
4821-96-04	TdT dNTP Mix	35 ml												•
4821-96-05	TdT Enzyme	35 ml												•
4821-96-14	50X Mn <sup>2+</sup>	100 µl												•
4822-96-08	TACS-Sapphire™	10 ml												•
4828-30-04	TACS™ B-dNTP Mix	30 µl		•				•	•					
4828-30-06	Anti-BrdU Antibody	30 µl		•				•	•					
4828-30-12	Strep-Diluent	7.5 ml		•				•	•					
4876-05-01	Cytonin™	5 ml	•	•		•	•	•		•	•	•		
4876-60-01	Cytonin™	6 ml											•	•
4800-30-09	DAB Enhancer	1 ml			•			•	•	•				
4800-30-18	Methyl Green	50 ml				•		•	•		•			
			<b>TISSUE SPECIFIC TACS™</b>					<b>ALL PURPOSE TACS™</b>					<b>SPECIALTY</b>	

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## WHAT CUSTOMERS SAY

Prof. Francesco D'Agostini  
Associate Professor  
Department of Health Sciences  
University of Genova Via A. Pastore  
Genova, Italy

*"The TACS XL<sup>®</sup> Blue Label and DermaTACS™ kits are a valuable tool for apoptosis detection: all the reagents are included, ready to use or very simple to prepare and the instructions are straightforward. The results are clear and unequivocal: blue-labeled apoptotic cells are well distinguishable and the background is very low. "*

Keith R. Brunt  
Ph.D Candidate  
Queen's University  
Kingston, Ontario, Canada

*"Trevigen's CardioTACS™ Kit provided a rapid and accurate assessment of tissue sections for the determination of apoptosis, as well as sufficient reagent for positive and negative control sections. The procedure is simple, reliable and reproducible with no batch variations between kits in our hands."*

Dr. Yang-Yi Fan  
Associate Research Scientist  
Texas Agricultural Experiment Station  
Texas A&M University

*"We have tried different kits from various vendors to detect apoptosis in mouse colon tissues. Trevigen's TACS™ 2 TdT-DAB kit gave us the best reproducible result.."*



## FAQS

- 1. Is it recommended that Trevigen NF Mounting Media (cat# 4865-25) be used with TACS™ In Situ Apoptosis Detection kits using either diaminobenzidine or TACS Blue Label™? Is it possible to use mounting media from other companies?**

It is important to use Mounting Media free of ortho-, para- and mixed xylenes. Xylenes result in fading of TACS Blue Label™. It is possible that fading of TACS Blue Label™ may occur with some batches of Permout due to presence of xylenes. DPX is advertised as free of xylenes. An alternate is butyl-acetate products from Polysciences, which is fast drying and is more likely to hold the color of the TACS Blue Label™.

# FAQS

## 2. What would cause my TACS Blue Label™ (cat# 4800-30-11) to appear green?

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The green appearance is due to oxidation (presence of bleach, metals, and other oxidizing agents.) Make sure to wash in deionized water before and after the TACS Blue Label™ (cat # 4800-30-11) step. Ensure work bench, pipettes, tips, etc are not contaminated with bleach or other strong oxidizing agents. Methyl Green may have been mistaken for TACS Blue Label™. The methyl green can look blue prior to the ethanol washes and fades to green as excess dye washes away.

## 3. What would cause my TACS Blue Label™ (cat# 4800-30-11) to fade or appear very weak?

---

Chlorine in tap water dissolves the TACS Blue Label™ hence it is essential to use deionized water. Ensure proper dehydration through decreasing alcohol series (ethanol or denatured ethanol only), and o- or p-xylenes only (no mixed xylenes). Make sure to change solutions frequently. Use correct mounting medium. Trevigen NF Mounting Media (cat# 4865-25) is the recommended mounting medium. The fading could also be due to benzene solubility. Benzene contaminants are found in some mixed xylenes. Use o- or p-xylenes for clarification after dehydration. Do not dilute mounting medium with mixed xylenes. Slides should be stored in the dark to maintain optimal staining. Insufficient wash steps can make the TACS Blue Label™ appear very weak. Make sure to wash in deionized water before and after the TACS Blue Label™ step. Labeling reaction time could also be insufficient. View under microscope to determine proper incubation period with TACS Blue Label™ (2-7 minutes).

## 4. What is the difference between Cytonin™ (cat# 4876-05-01) and Proteinase K (cat# 4800-100-01)?

---

Customers should use either Proteinase K (cat# 4800-100-01) or Cytonin™ (cat# 4876-05-01) for cell permeabilization. Proteinase K is a robust permeabilization reagent and can compromise cell membrane integrity with long incubation periods. Cytonin™ is much gentler but may require optimization for some cell types and tissues.

## 5. Which method or kit is recommended to detect apoptosis in frozen brain (hippocampus) samples from a heat stressed animal? Are there any pre-treatments for thick tissue sections? Is a free floating method required?

---

NeuroTACS™ II In Situ Apoptosis Detection Kit (cat# 4823-30-K) is recommended for convenient identification of apoptosis in brain tissue or neuronal cells. Fresh frozen tissue needs to be fixed on a slide and permeabilized using NeuroPore™ (cat# 4820-30-01). With some thick tissue samples, only the top layer of the sample may be labeled. For fixed frozen tissues, the free floating method may be used to ensure labeling on both sides of the sample.

# RELATED PRODUCTS

Researchers who purchased TACS™ products also purchased these...

## TACS™ DNA Laddering Kits

The TACS™ Apoptotic DNA Laddering Kits are used to detect and estimate the level of internucleosomal DNA fragmentation that occurs during apoptosis. Kit selection is dependent upon the degree of apoptosis, number of cells and availability of equipment. The evidence of DNA laddering supports other experimental data derived from morphological identification methods. Each kit contains all reagents necessary to isolate, label and detect DNA.

Product Description	Label	Size	Catalog No.
Apoptotic DNA Laddering Kit	Ethidium Bromide	20 Samples	4850-20-ET
Apoptotic DNA Laddering Kit	Isotopic	20 Samples	4850-20-K
Apoptotic DNA Laddering Kit	Chemiluminescent	20 Samples	4855-20-K
Apoptotic DNA Laddering Kit	Colorimetric	20 Samples	4857-20-K

## PARP/Apoptosis Kits

Trevigen's PARP/Apoptosis Kits are ideal for measuring the activity of PARP in cell and tissue extracts before, during and after apoptosis. During apoptosis, PARP-1 which catalyzes the NAD dependent addition of poly (ADP-ribose) (PAR) onto various cytoplasmic and nuclear proteins, is cleaved from its enzymatically active form to its inactive form. The HT PARP/Apoptosis Assay is an ELISA which detects PAR deposited onto immobilized histone proteins (via PARP activity) in a strip well format.

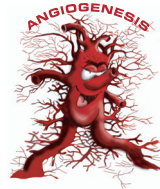
Product Description	Detection	Size	Catalog No.
HT Colorimetric PARP/Apoptosis Assay	Colorimetric	96 Tests	4684-096-K
HT Chemiluminescent PARP/Apoptosis Assay	Chemiluminescent	96 Tests	4685-096-K



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