TREVIGEN® Instructions

For Research Use Only. Not For Use In Diagnostic Procedures

HT TiterTACS™ Assay Kit

Quantitative Assay for the Detection of Apoptosis in Cells

96 Well Colorimetric Kit

Sufficient reagents for:
96 tests-96well format

Cat #: 4822-96-K

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TACS (**T**revigen **A**poptotic **C**ell **S**ystem

HT TiterTACS™

I. Quick Reference Procedure

Read through the complete *Instructions for Use* before following this protocol. Some incubation times and washes may be altered based on results obtained using controls. Use between 2 x 10^4 and 1 x 10^5 cells/well, fixed and washed in PBS. See section VI. A. Reagent Preparation. Note: a) Centrifuge at 1000 x g for 3 minutes at room temperature between steps. b) Perform all steps at room temperature unless otherwise noted.

This page is designed to be photocopied and used as a checklist.

- ☐ 1. Wash cells with 200 µl of 1X PBS per well, centrifuge and discard PBS.
- ☐ 2. Add 50 µl of Proteinase K solution per well. Incubate for 15 minutes.

volume/sample:	volume/100 samples:
☐ 50 µl dH_2O	☐ 5 ml dH_2O
☐ 1 µl Proteinase K	☐ 100 µl Proteinase K

- ☐ **OR** add 50 µl of Cytonin™ per well and incubate for 30 minutes.
- ☐ 3. Centrifuge and discard Proteinase K solution or Cytonin™.
- ☐ 4. Wash with 200 µl dH_2O per well.
- ☐ 5. Add 50 µl of hydrogen peroxide solution per well.

volume/sample:	volume/100 samples:
☐ 45 µl Methanol	☐ 4.5 ml Methanol
☐ 5 µl 30% H_2O_2	☐ 500 µl 30% H_2O_2

- ☐ 6. Centrifuge and discard hydrogen peroxide solution.
- ☐ 7. Add 150 ul of 1X TdT Labeling Buffer per well.

volume/sample:	volume/100 samples:
☐ 200 µl dH_2O*	☐ 20 ml dH_2O
☐ 20 µl 10X TdT Labeling buffer	☐ 2 ml 10X TdT Labeling buffer

This step prepares enough buffer to retain 50 µl per sample for step 9.

- ☐ 8. Centrifuge and discard buffer.
- ☐ 9. Add 50 µl of Labeling Reaction Mix per well. Incubate for 60 minutes at 37 °C in a humidity chamber.

volume/sample:	volume/100 samples:
☐ 50 µl 1X TdT Labeling Buffer	☐ 5 ml 1X TdT Labeling Buffer
☐ 1µl Mn^{2+}	☐ 100 µl Mn^{2+}
☐ 0.35 µl TdT dNTP mix	☐ 35 µl TdT dNTP mix
☐ 0.35 µl TdT Enzyme	☐ 35 µl TdT Enzyme

- ☐ 10. Stop the reaction with 150 ul of 1X TdT Stop Buffer per well.

volume/sample:	volume/100 samples:
☐ 150 µl dH_2O	☐ 15 ml dH_2O
☐ 15 µl 10X TdT Stop Buffer	☐ 1.5 ml 10X TdT Stop Buffer

- ☐ 11. Centrifuge and discard stop buffer.
- ☐ 12. Wash wells two times with 200 µl of 1X PBS per well.
- ☐ 13. Add 50 µl of Strep-HRP Solution per well. Incubate for 10 minutes.

volume/sample:	volume/100 samples:
☐ 50 µl Blue Strep-diluent	☐ 5 ml Blue Strep-diluent
☐ 0.04 µl Strep-HRP	☐ 4 µl Strep-HRP

- ☐ 14. Centrifuge and discard Strep-HRP solution.
- ☐ 15. Wash four times with 200 µl of PBS, 0.1% Tween 20 per well.
- ☐ 16. Add 100 µl of TACS-Sapphire™ per well.
- ☐ 17. Incubate plate at room temperature for 15-60 minutes in the dark.
- ☐ 18. Measure reaction kinetics at 630 nm. Add 100 µl of 5% phosphoric acid OR 0.2N HCl per well to stop reaction and record optical density at 450 nm.

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II. Background

The **TACS** (**T**revigen **A**poptotic **C**ell **S**ystem) *in situ* kits allow identification of apoptotic cells using a variety of formats. For many cell types in culture, identification of apoptosis can be achieved using a combination of morphological criteria, extraction and analysis of DNA by agarose gel electrophoresis, and *in situ* detection of DNA fragmentation in immobilized cells. Other approaches include measuring the activation of ICE-like proteases, detection of annexin binding at the cell surface and activation of poly-ADP ribose polymerases. However, in many cases, the treatment and analysis of a large number of samples is inconvenient. TiterTACS™ allows quantitative colorimetric analysis of a large number of cell samples using a 96-well plate. The assay provides quantitation of apoptosis in cultured cells without direct counting of labeled cells. Trevigen's **HT TiterTACS™** is a reagent kit designed specifically for quantitation of apoptosis in suspension and mono-layer cell cultures. The kit is derived from Trevigen's **TACS 2 TdT *In Situ* Apoptosis Detection Kit** and employs *in situ* colorimetric detection. **HT TiterTACS™** provides all the reagents for the detection of DNA fragmentation in cells grown as a monolayer or in suspension. **TACS-Nuclease™** allows positive controls to be generated for each experimental system: a brief treatment of cells with TACS-Nuclease™ prior to labeling generates DNA breaks in every cell, providing an appropriate positive control specific for the system under study. Contact Trevigen for information on additional Trevigen products available for the study of apoptosis, cell proliferation DNA damage, and DNA repair.

The **HT TiterTACS™** 96 well plate format offers:

- A sensitive and easy to follow assay
- Cost-effective screening of up to 96 different samples for apoptosis/kit
- Quantitative read-out, allowing for statistical analysis

III. Precautions and Limitations

1. For Research Use Only. Not for use in diagnostic procedures.
2. The physical, chemical and toxicological properties of these products may not yet have been fully investigated therefore Trevigen recommends the use of gloves, lab coats and eye protection while using these chemical reagents. Trevigen assumes no liability for damage resulting from hand-ling or contact with these products.

IV. Materials Supplied

Component	Amount Provided	Storage	Cat#
Cytonin™	6 ml	4 °C	4876-60-01
Proteinase K	100 µl	-20 °C	4821-96-01
10X TdT Labeling Buffer	20 ml	4 °C	4817-60-02
10X TdT Stop Buffer	20 ml	4 °C	4817-60-03
TdT dNTP Mix	35 µl	-20 °C	4821-96-04
TdT Enzyme	35 µl	-20 °C	4821-96-05
50X Mn^{2+}	100 µl	-20 °C	4821-96-14
Strep-HRP	30 µl	4 °C	4800-30-06
Blue-Strep diluent	7.5 ml	4 °C	4800-30-12
TACS-Sapphire™	10 ml	4 °C	4822-96-08
TACS-Nuclease™	15 µl	-20 °C	4800-30-15
TACS-Nuclease™ Buffer	1.5 ml	4 °C	4800-30-16

V. Materials required but not supplied

Equipment:	Reagents:	Disposables:
1-20 µl pipettor	Apoptosis Grade™ Water•	96 well microplate
20-200 µl pipettor	100% ethanol **or** denatured alcohol	Microcentrifuge tubes
1-50 µl multichannel pipettor	10X PBS•	1-200 µl pipette tips
50-300 µl multichannel pipettor	Tween 20	10 ml serological pipettes
Humidity chamber	37% Formaldehyde	15 ml tubes
37 °C incubator	Sucrose	
-20 °C and 4 °C storage	30% Hydrogen Peroxide	
Microplate reader	Methanol	
Centrifuge with microplate adapters	5% phosphoric acid or 0.2N HCl	

•Available from Trevigen. Please See Related Products (pages 10-12).

VI. Reagent Preparation

Reagents marked with an asterisk (*) should be prepared immediately before use.

The volumes given for each reagent are based on processing samples in a 96 well plate. If conical well plates are used, the volume of solution may be decreased to 30 µl per well.

1. **1X PBS** Please see page 11 to order or prepare 10X PBS. Approximately 100 ml of 1X PBS is used to process 96 samples. Dilute 10X PBS to 1X using distilled water. Store 1X PBS at room temperature.

2. ***3.7% Buffered Formaldehyde**
   20 ml of fixative is used to process 96 samples.

To prepare:	
sucrose	10 g
1X PBS	40 ml
37% Formaldehyde	5 ml
adjust volume with 1 X PBS to	50 ml

   Store at room temperature. Wear gloves and exercise caution when handling formaldehyde solutions.

3. ***Proteinase K solution**
   50 µl of Proteinase K Solution is used per sample. Store on ice. Thaw Proteinase K at room temperature and then place on ice. Prepare immediately before use. To prepare add:

	1 sample	100 samples
dH_2O	50 µl	5 ml
Proteinase K (Cat# 4821-96-01)	1 µl	100 µl

4. **Cytonin™ - Cat# 4876-60-01**
   If required, 50 µl of Cytonin™ is used per sample.
   Cytonin™ is ready to use. Store at 4˚C. Discard if cloudy.

5. ***3% Hydrogen Peroxide solution**
   5 ml of hydrogen peroxide solution is necessary per 100 samples.
   To prepare 6 ml of solution mix:
   0.5 ml 30% Hydrogen peroxide
   5.5 ml methanol

6. **1X TdT Labeling Buffer**
   25 ml of 1X Labeling Buffer is enough to process 96 samples. Dilute the 10X TdT Labeling Buffer (Cat# 4817-60-02) to 1X using distilled water. Leave at room temperature until use. Remove an aliquot of 50 µl of 1X labeling buffer per sample to prepare the Labeling Reaction Mix, and place on ice.

7. ***Labeling Reaction Mix**
   Thaw TdT dNTP Mix (Cat# 4821-96-04) at room temperature and then place on ice. To maintain optimal activity, remove the TdT Enzyme (Cat# 4821-96-05) tube from the freezer only to pipette the required volume. Alternatively, place the TdT Enzyme in a -20˚C freezer block. Prepare the Labeling Reaction Mix just before use and keep the prepared reaction mix on ice. Prepare one sample without the enzyme (refer to Section VIII on controls).

   Prepare 50 µl per sample:

	100 samples	n samples
1X TdT Labeling Buffer (Step 6)	5 ml	n x 50 µl
TdT dNTP Mix (Cat# 4821-96-04)	35 µl	n x 0.35 µl
50X Mn^{2+} (Cat# 4821-96-14)	100 µl	n x 1 µl
TdT Enzyme (Cat# 4821-96-05)	35 µl	n x 0.35 µl

8. **1X TdT Stop Buffer**
   20 ml of 1X TdT Stop Buffer is used to process 96 samples.
   Dilute 2 ml of 10X TdT Stop Buffer (Cat# 4817-60-03) to 1X with 18 ml dH_2O.
   Leave at room temperature until use.

9. ***Strep-HRP Solution**
   50 µl of Strep-HRP Solution (1:1250) is used per sample.

	100 samples	n samples
Blue-Strep Diluent (Cat# 4800-30-12)	5 µl	n x 50 µl
Strep-HRP (Cat# 4800-30-06)	4 µl	n x 0.04 µl

10. **TACS-Sapphire™ (Cat# 4822-96-08)**
    Use substrate solution at room temperature, add 100 µl of solution per well.
    TACS-Sapphire™ is ready to use. Protect from light.

11. ***TACS-Nuclease™ Solution**
    50 µl of Nuclease Solution is required for each Nuclease-treated control sample.
    Avoid repeated freeze-thaw of TACS-Nuclease™. To prepare add:

	1 sample	n samples
TACS-Nuclease™ Buffer (Cat# 4800-30-16)	50 µl	n x 50 µl
TACS-Nuclease™ (Cat# 4800-30-15)	1 µl	n x 1 µl

    Prepare Nuclease Solution just before use and place on ice.

12. **Stop solution**
    5% Phosphoric Acid or 0.2N HCl
    add 100 µl per well to stop the colorimetric reaction.

***Reagents should be prepared immediately before use.**

VII. Assay Protocol

It is important to read through the "Instructions for Use" before preparing cell samples for labeling. There are key steps that are very important for successful labeling. This section includes instructions for Sample Preparation, *In Situ* Labeling and plate reading. The Assay Protocol for Labeling is in tabulated form and details the steps involved in the labeling reaction. Prior to labeling, the samples must be fixed and washed in PBS. The labeling procedure begins with samples in PBS regardless of the fixation and immobilization method.

A. Sample Preparation and Fixation

1. Preparation of Suspension Cells

Cells grown in suspension, or prepared from dissociated tissues, can be fixed in batch solution and then transferred to 96 well plates or may be grown and fixed directly in the 96 well plates.

Batch Method

1. Harvest cell suspension by centrifugation at 500 x g for 5 minutes at room temperature. Prepare enough cells for your assay. Typically between 2 x 10^4 to 1 x 10^5 cells/well will generate sufficient signal.
2. Wash in 1X PBS and centrifuge.
3. Discard PBS and resuspend at 1 x 10^6 cells/ml in 3.7% Buffered Formaldehyde solution (See section VI, Reagent Preparation). Let stand for 7 minutes at room temperature. (Do not leave longer than 10 minutes).
4. Centrifuge at 500 x g for 5 minutes at room temperature and discard fixative.
5. Wash once with 1X PBS and centrifuge.
6. Post-fix sample in 100% methanol for 20 minutes at room temperature.
7. Wash cell pellet twice in 1X PBS. Centrifuge between washes.
8. Resuspend cells at 1 x 10^6 cells/ml in 1X PBS
9. Distribute cells at 2 x 10^4 - 1 x 10^5 cells/well. The exact cell number should be determined empirically.
10. Proceed to Section VII.B., Labeling Procedure.

In Well Method

1. Distribute or grow 2 x 10^4 to 1 x 10^5 cells/well in a 96 well plate.
2. Centrifuge plate at 1000 x g for 3 minutes at room temperature and discard media.
3. Wash once with 1X PBS at room temperature.
4. Fill wells with 3.7% Buffered Formaldehyde solution. Let stand for 7 minutes at room temperature (Do not leave longer than 10 minutes).
5. Centrifuge plates at 1000 x g for 3 minutes at room temperature and discard fixative.
6. Wash cells with 1X PBS.
7. Post fix sample in 100% methanol for 20 minutes at room temperature.
8. Wash wells twice with 1X PBS. Centrifuge between washes.
9. Proceed to Section V.B., Labeling Procedure.

2. Preparation of Cells in Monolayer Method

1. Centrifuge plate at 1000 x g for 3 minutes at room temperature and discard the media.
2. Wash two times with 1X PBS at room temperature. Centrifuge between washes.
3. Fill wells with 3.7% Buffered Formaldehyde solution. Let stand for 7 minutes at room temperature (Do not leave longer than 10 minutes).
4. Centrifuge as in step 1 and discard fixative.
5. Wash twice with 1X PBS at room temperature. Centrifuge between washes.
6. Post fix sample in 100% methanol for 20 minutes.
7. Wash wells two times with 1X PBS. Centrifuge between washes.
8. Proceed to Section VII.B., Labeling Procedure.

3. For Storage Options Please See Appendix C (page 12)

B. Labeling procedure for hydrated fixed cells in 96 well plates:

Step	Instructions	Notes
1	Add 50 µl of Proteinase K solution (page 3) per well and incubate for 15 minutes at room temperature.	Samples can also be treated with 50 µl/well of Cytonin™ as an alternative to Proteinase K. Cytonin™ is recommended for cells in monolayer.
2	Centrifuge plate at 1000 X g for 3 min. at room temperature and discard the buffer.	
3	Wash once with 200 µl/well of dH_2O.	Centrifuge plate between each wash (as in step 2).
4	Generate a positive control using TACS-Nuclease™ at this point. Other samples may be covered with PBS during preparation of the positive nuclease treated control.	After dH_2O washes, add 50 µl of TACS-Nuclease™ Solution (page 4) to each control well. Incubate for 10-60 minutes at 37°C.
5	Wash samples for 2 minutes in PBS.	Centrifuge plate between each wash.
6	Quench endogenous peroxidase. Add 50 µl/well peroxide solution (page 3) and incubate for 5 minutes at room temperature.	Do not exceed 5 minutes.
7	Wash once with 200 µl/well of dH_2O.	Centrifuge plate between each wash.
8	Add 150 µl/well of 1X TdT Labeling Buffer (page 4). Leave for 5 minutes.	
9	Centrifuge plate and discard buffer.	
10	Add 50 µl/well of Labeling Reaction mix (page 4) and incubate at 37°C for 1 hour.	Use a humidity chamber (see Appendix D) or a microplate cover during incubation.
11	Add 150 µl/well of 1X TdT Stop Buffer (page 4) for 5 minutes to stop labeling reaction.	
12	Centrifuge plate and discard buffer.	
13	Wash samples twice with 1X PBS for 2 minutes per wash.	Centrifuge plate between each wash.
14	Add 50 µl/well of Strep-HRP Solution (page 4) and incubate at room temperature for 10 min.	
15	Wash samples four times with 200 µl/well of PBS, 0.1% Tween 20.	Centrifuge plate between each wash.
16	Add 100 µl/well of TACS-Sapphire (page 4) at room temperature.	If working with suspension cells, ensure that cells are resuspended.
17	Incubate at room temperature for 30 minutes in the dark.	Follow kinetics of the reaction at 630 nm to determine linear range.
18	Stop reaction with 100 µl of 5% phosphoric acid or 0.2 N HCl per well. Measure absorbance at 450 nm.	Read plate within 30 minutes of acid addition.

VIII. Controls

The controls that should be included, especially when performing the protocol for the first time, are listed below. Controls should be performed in duplicates or triplicates.

TACS Nuclease™-treated control

Treat two or three samples with TACS-Nuclease™ to generate DNA breaks in every cell. Avoid repeated freeze-thaw cycles. The TACS Nuclease™-treated controls will confirm that the permeabilization and labeling reaction has succeeded. The information can help optimize the conditions for the labeling procedure. The colorimetric readings obtained with this control will be higher than the experimental values and will provide a maximum value.

Unlabeled Experimental Control Sample

The TdT enzyme should be omitted from the Labeling Reaction Mix for two or three samples. These controls will indicate the level of background labeling associated with non-specific binding of the Strep-HRP. These controls should have low or negligible absorbance.

Experimental Negative Control Sample

An appropriate experimental control should be included in each experiment and will depend upon the system under study. Typically the Experimental Negative Control will be an untreated sample, or normal cells. Many normal or untreated cells and tissues will have a small number of apoptotic cells, resulting in a low level of labeling.

IX. Data Interpretation

Duplicate or triplicate samples will allow statistical validation of results. The Controls listed in Section VIII are important in data interpretation and allow optimization of *In Situ* detection of apoptosis without expending valuable test samples. Refer to the Troubleshooting Guide for information if the Controls do not provide the expected result.

Figure 1 and Figure 2 show typical results obtained with TiterTACS™:

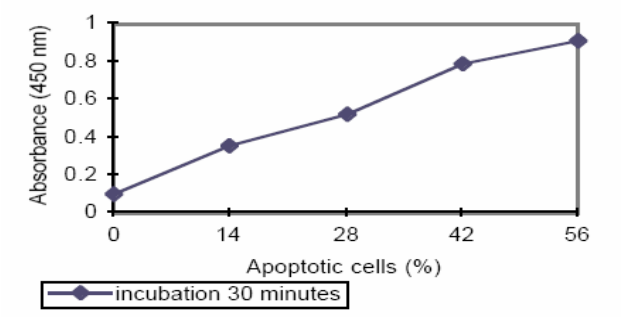

Figure 1 Quantitation of Apoptosis in Staurosporine-treated ML-1 cells using HT TiterTACS™.

Detection of apoptosis in fixed ML-1 cells after treatment with 1 mM staurosporine for 24 hours. Cells were harvested, fixed and labeled according to the HT TiterTACS™ protocol prior to colorimetric analysis. Cells were incubated with TACS-Sapphire™ substrate and the colorimetric reaction was stopped with 0.2N HCl after 30 minutes. The percentage of apoptotic cells in the culture was estimated by enumeration using the TACS 2 TdT-DAB™ *in situ* labeling kit. The cell culture was diluted with a non-apoptotic cell culture to obtain the different concentration of apoptotic cells for the assay.

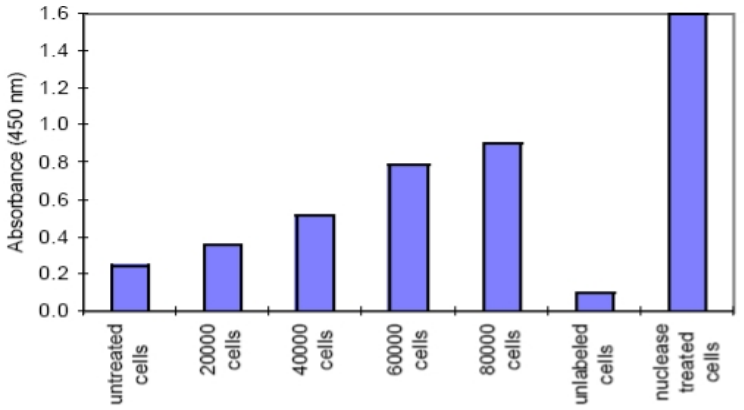

Figure 2 Quantitation of Apoptosis in Staurosporine Treated ML-1 Cells using HT TiterTACS™.

Data obtained after stopping the reaction with 50% phosphoric acid, 30 minutes after addition of substrate. Control wells were untreated (without apoptosis inducer), unlabeled (with out TdT enzyme) and nuclease treated cells. All the control wells contained 100 000 cells.

Note: Experimental results may vary depending on the type of cells, cell treatment, incubations and storage conditions. Morphological observation of the cells is recommended prior to assay.

X. Troubleshooting Guide

Rule out major problems by checking the labeling in the control samples first.

Problem	Cause	Action
No labeling in TACS-Nuclease™ treated sample.	Poor permeabilization and/or excesssive fixation with cross-linking fixative preventing enzyme access.	Optimize Proteinase K treatment or optimize time in Cytonin™, reduce time in fixative to 5 minutes.
	No DNA left in sample due to hydrolysis (poor storage of samples).	Please read Appendix C prior to labeling.
	Excessive (removed all DNA) or inadequate Nuclease treatment.	Optimize time for Nuclease treatment (5 minutes up to 2 hours).
	TdT Enzyme is inactive. The enzyme is the most labile component in the kit.	TdT Enzyme must be stored at -20 °C. Do not bring enzyme up to ice temperature. Place in -20 °C block or remove aliquot from tube directly in freezer. Store in manual defrost freezer.

Problem	Cause	Action
No labeling in experimental sample.	No apoptosis (or necrosis) occurring in sample.	If all controls gave the expected results and were processed at the same time as the experimental sample there may be no DNA fragmentation in cells within the sample. Always examine the morphology of cells.
Excessive background in negative control.	Residual unlinked Strep-HRP. Non-specific binding of Strep-HRP.	Wash cells at least 4 times with PBS, 0.1% Tween 20. Incubate Strep-HRP with a blocking reagent such as 5% (w/v) non-fat dried milk or fetal bovine serum in PBS, 0.1% Tween® 20.
Poor duplicate or triplicate values.	Non-sufficient centrifugation Poor removal of buffer. Loss of cells after or during washes.	Centrifuge after every wash. Use care when pipetting off buffer. Use conical 96 well plate to perform assay on suspension cells: transfer to flat bottom plate after incubation with TACS-Sapphire.

Tween® 20 is a registered trademark of ICI Americas, Inc. Wilmington, DE.

XI. References

Books:

Apoptosis: The Molecular Basis of Cell Death. Current Communications in Cell and Molecular Biology, Vol 3. Ed. L. D. Tomei and F. O. Cope (1991) Cold Spring Harbor Laboratory Press, New York.

Apoptosis II: The Molecular Basis of Apoptosis in Disease. Current Communications in Cell and Molecular Biology, Vol 8. Ed. L. D. Tomei and F. O. Cope (1994) Cold Spring Harbor Laboratory Press, New York.

Articles:

S.-R. Shi, R.J. Cote, L.L. Young and C.R. Taylor (1997) Antigen retrieval immunohistochemistry: practice and development. *J. Histotechnology* **20**: 145 - 154.

A. Negoescu, P. Lorimier, F. Labat-Moleur, C. Drouet, C. Robert, C. Guillermet, C. Brambilla, E. Brambilla,(1996). *In situ* apopototic cell labeling by the TUNEL method: improvements and evaluation on cell preparation. *J. Histochem. Cytochem.* **44**:959-968.

J. F. Kerr, G. C. Gobe, C.M. Winterford and B.V. Harmon (1995) Anatomical methods in cell death. *Methods in Cell Biology* **46**: 1 - 27.

M. Yamawaki, A. Zurbriggen, A. Richard and M. Vandevelde (1993) Saponin treatment for *in situ* hybridization maintains good morphological preservation. *J. Histochem. Cytochem.* **41**: 105 - 109.

P. Seubert, C. Vigo-Pelfrey, F. Esch, M. Lee, H. Dovey, D. Davis, S. Sinha, M. Schlossmacher, J. Whaley and C. Swindlehurst (1992) Isolation and quantitation of soluble Alzheimer's b-peptide from biological fluids. *Nature* **359:** 325-327.

XII. Related products available from Trevigen.

Catalog #	Description	Size
4817-60-K	FlowTACS™ Kit	60 samples
4830-01-K	TACS™ Annexin V FITC Kit	100 samples
4835-01-K	Annexin V Biotin Kit	100 samples
6300-100-K	DePsipher™ Mitochondrial Potential Assay Kit	100 tests
6305-100-K	MitoShift™ Mitochondrial Potential Assay Kit	100 tests
4815-30-K	TumorTACS™ *In Situ* Apoptosis Detection Kit	30 samples
4823-30-K	NeuroTACS™ *In Situ* Apoptosis Detection Kit	30 samples
4827-30-K	CardioTACS™ *In Situ* Apoptosis Detection Kit	30 samples
4829-30-K	DermaTACS™ *In Situ* Apoptosis Detection Kit	30 samples
4826-30-K	VasoTACS™ *In Situ* Apoptosis Detection Kit	30 samples
4828-30-DK	TACS•XL® DAB *In Situ* Apoptosis Detection Kit	30 samples
4828-30-BK	TACS•XL® Blue Label *In Situ* Apoptosis Detection Kit	30 samples
4810-30-K	TACS™ 2 TdT DAB *In Situ* Apoptosis Detection Kit	30 samples
4811-30-K	TACS™ 2 TdT Blue Label *In Situ* Apoptosis Detection Kit	30 samples
4812-30-K	TACS™ 2 TdT Fluorescein Apoptosis Detection Kit	30 samples
4850-20-ET	TACS™ Apoptotic DNA Laddering Kit EtBr	20 samples
4855-20-K	TACS™ Apoptotic DNA Laddering Kit Chemiluminescent	20 samples
4857-20-K	TACS™ Apoptotic DNA Laddering Kit Colorimetric	20 samples
4850-20-K	TACS™ Apoptotic DNA Laddering Kit Isotopic	20 samples
4895-50-K	TACS™ 2 Hoechst CPA1 Kit (Vital)	2500 tests
4896-50-K	TACS™ 2 Hoechst CPA2 Kit (Fixed)	2500 tests
4892-010-K	Cultrex® Calcein-AM Cell Viability Kit	1000 tests
4667-50-K	PARP Activity Assay Kit	50 tests
4671-096-K	HT Universal Color PARP Assay Kit/w Histone Reagents	96 samples
4675-096-K	Universal Chemiluminescent PARP Assay Kit/w Histone Reagent	96 samples
4676-096-K	Universal Chemiluminescent PARP Assay Kit/w Histone Coated Strip Wells	96 samples
4677-096-K	Universal Color PARP Assay Kit/w Histone Coated Strip Wells	96 samples
4667-250-01	Recombinant Human PARP Enzyme	250 µl
4668-100-1	Recombinant Human PARP (High Specific Activity)	1000 Units
4682-096-K	HT Chemiluminescent PARG Assay Kit	96 tests
4683-096-K	HT Colorimetric PARG Assay Kit	96 tests
6218-020-K	Caspases 3; 8; 9 Inhibitors Kit	20 µl each
4667-50-11	Benzamide PARP inhibitor (8 mM)	100 µl
4667-50-10	6(5H)-Phenanthridinone PARP inhibitor (160 µM)	100 µl
4667-50-9	4-Amino-1,8-naphthalimide PARP inhibitor (800 µM)	100 µl

Antibodies

Catalog #	Description	Size
4411-PC-100	Anti-Phosphorylated Histone-γ-H2AX polyclonal	100 µl
2341-PC-050	Anti-human/mouse-Bim	50 µg
2315-PC-050	Anti-human-Bid polyclonal	50 µg
2316-PC-075	Anti-human-Mcl-1 polyclonal	75 µg
2317-PC-050	Anti-human-TRAIL polyclonal	50 µg
2318-PC-075	Anti-human-cIAP-2 polyclonal	75 µg
2319-PC-050	Anti-human-cIAP-1 polyclonal	50 µg
2321-PC-100	Anti-human-Drp-1 polyclonal	100 µg
2323-PC-050	Anti-human/murine-XIAP polyclonal	50 µg
2340-MC-050	Anti-human-APAF-1 (clone 94408.11)	50 µg

Catalog #	Description	Size
2342-PC-050	Anti-human-CD95 (Fas) polyclonal	50 µg
2343-MC-250	Anti-human-CD95 (Fas) mAb (clone Dx2)	250 µg
2344-MC-250	Anti-human-CD95 (Fas) mAb (clone 50830)	250 µg
2290-MC-100	Anti-murine-Bcl-2 mAb (clone YTH-10C4)	100 µg
2291-MC-100	Anti-human-Bcl-2 mAb (clone YTH-8C8)	100 µg
2300-MC-100	Anti-human-Bcl-XL mAb (clone YTH-2H12)	100 µg
2330-MC-025	Anti-human-Bax and –Bcl-2 mAbs	25 µg each
2280-MC-100	Anti-mouse-Bax mAb (clone YTH-5B7)	100 µg
2281-MC-100	Anti-human-Bax mAb (clone YTH-6A7)	100 µg
2282-MC-100	Anti-human-Bax mAb (clone YTH-2D2)	100 µg
2304-PC-040	Anti-human/mouse-Caspase 2 polyclonal	40 µg
2305-PC-100	Anti-cleaved human/mouse-Caspase 3 polyclonal	40 µg
2308-MC-050	Anti-human-Caspase 9 mAb	50 µg
2309-MC-050	Anti-human/mouse-Caspase 10 mAb	50 µg
2310-PC-050	Anti-human/mouse-Caspase 10 polyclonal	50 µg
2312-MC-050	Anti-human-Caspase 7 mAb	50 µg
6360-PC-100	Anti-human/mouse-PBR polyclonal	100 µl
6370-MC-100	Anti-human/murine-Cytochrome C	100 µg
6380-MC-100	Anti-human/murine-Holocytochrome C	100 µg
4335-MC-100	Anti-PAR polymer mAb (10HA)	100 µl
4336-BPC-100	Anti- PAR polymer polyclonal	100 µl
4338-MC-50	Anti-human/murine-PARP mAb (clone C2-10)	50 µg

Accessories

Catalog #	Description	Size
4800-30-40	Tissue Control Slides	2 ea.
4800-30-20	Cell Culture Control Slides	2 ea.
4864-100	Treated Glass Microscope Slides w/3 sample Hydrophobic Barrier	100 ea.
4800-30-20	Cell Culture Control Slides	2 ea.
4867-100	Hydrophobic Coverslips	100 ea.
4862-10	Coverslips 24 x 60 mm, No. 1.5	2 ea.
4865-25	Mounting Medium	10 x 1 oz.
4800-30-14	Strep-Fluorescein	25 ml
4830-100-03	Propidium Iodide	30 µl
4870-500-6	10X PBS	6 x 500 ml
4869-500-6	Apoptosis Grade™ H_2O	6 x 500 ml
4820-30-13	Blue Counterstain	50 ml
4825-30-RL	Red Label™	5 ml
4878-05-02	Cytonin™ IHC	2 x 5 ml

XIII. Appendices

Appendix A. Reagent and Buffer Composition

10X PBS, pH 7.4 (Cat# 4870-500-6):
1.45 M sodium chloride
75 mM disodium hydrogen phosphate
25 mM sodium dihydrogen phosphate

Apoptosis Grade™ Water (Cat# 4869-500-6):
Deionized (18 mW) sterile water
The water used should be DNase free
Distilled autoclaved water can be used

Cytonin'™ (Cat# 4876-60-01):
Proprietary permeabilization agent

10X TdT Labeling Buffer (Cat# 4817-60-02):
1 M Safe-TdT Buffer™
0.5 mg/ml BSA
0.6 mM 2-Mercaptoethanesulfonic Acid

10X TdT Stop Buffer (Cat# 4817-60-03):
0.1 M EDTA, pH 8.0

TdT dNTP Mix (Cat# 4821-96-04):
0.25 mM Biotinylated dNTP
7.5 mM dNTPs

TACS-Nuclease™ (Cat# 4800-30-15):
Proprietary endonuclease. Avoid repeated freeze-thaw.

TACS-Nuclease™ Buffer (Cat# 4800-30-16):
50 mM Tris-HCl, pH 8.0
1 mM $MgCl_2$
100 mg/ml BSA

TACS-Sapphire™ (Cat# 4822-96-08):
Non-toxic, non-organic peroxidase substrate

Appendix B. Fixation Methods

There are several fixation methods commonly used that are appropriate for the protocol described in the Instructions for Use. Formaldehyde is the recommended fixative based on laboratory testing. However, other fixatives that maintain DNA integrity may be used. These include alcohol fixatives such as ethanol, methanol or acetone and other cross linking agents including paraformaldehyde and glutaraldehyde. Regardless of the fixative used, it is important not to fix cells for extended periods of time. Fixatives other than formaldehyde should be empirically tested to ensure cells can be labeled post-fixation.

Appendix C. Storage

a) Long term storage: After fixation and post-fixation steps, cells can be stored in 80% ethanol at 4 °C or -20 °C for several weeks or months. If cells are stored in a microplate, an adhesive or plastic cover is recommended to prevent contamination or evaporation. For labeling after storage, wash the cells with 80% ethanol, then wash three times with 1X PBS and proceed to Labeling Protocol (page 6). ***Note***: When cells are fixed using alcohol, *e.g.* ethanol, signal intensity in positive cells may diminish with time due to loss of small DNA fragments. b) Short term storage: The cells can be stored, prior to step 1 of the labeling reaction at 4 °C for periods up to a month. An adhesive plate cover is recommended to prevent contamination and evaporation.

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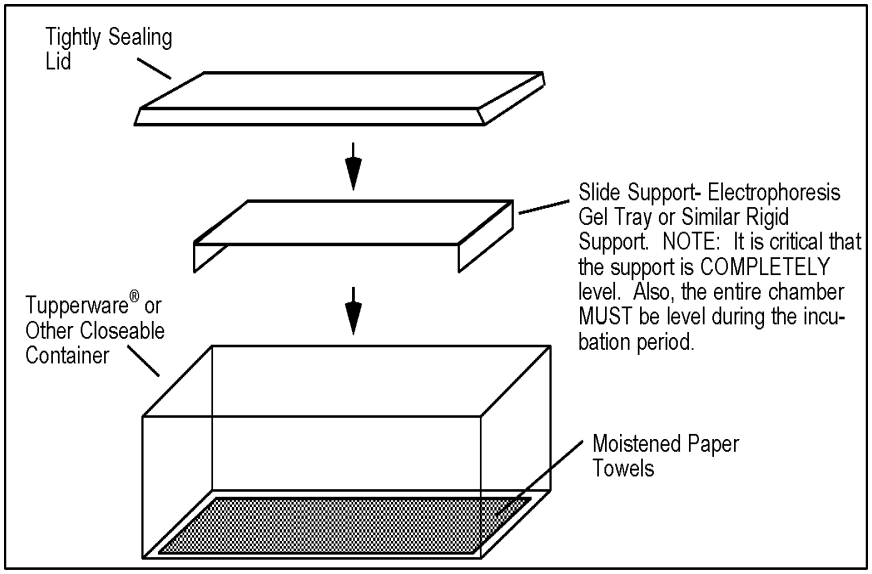
Appendix D. Humidity Chamber

To prevent evaporation it is recommended that incubations at 37 °C be carried out in a humidity chamber. A humidity chamber can be made using a plastic box with a tight fitting lid and two glass rods or other support. Place a paper towel on the bottom of the box and wet thoroughly with water. Lay the glass rods parallel to each other and less than one 96 well plate length apart on the wet tissue. Position the plate on the glass rods and place the plastic box, with lid, in a 37 °C incubator. Ensure that the plate is horizontal.



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Trevigen, Inc.
8405 Helgerman Ct. Gaithersburg, MD 20877
Tel: 1-800-873-8443 • 301-216-2800
Fax: 301-560-4973
e-mail: info@trevigen.com
www.trevigen.com

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