

DePsipher™ Kit

for the Detection of Mitochondrial
Membrane Potential ($\Delta\Psi_m$)

Catalog # 6300-100-K

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100 Tests

I. Background

Cellular energy produced during mitochondrial respiration is stored as an electrochemical gradient across the mitochondrial membrane. This accumulation of energy in healthy cells creates a mitochondrial trans-membrane potential, called delta-psi or $\Delta\Psi_m$, that enables the cell to drive the synthesis of ATP. Disruption of $\Delta\Psi_m$ has been shown to be one of the first intracellular changes following the onset of apoptosis. Trevigen's DePsipher™ Kit uses a unique cationic dye (5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazolylcarbocyanine iodide) to indicate the loss of the mitochondrial potential. The dye readily enters cells and fluoresces brightly red in its multimeric form within healthy mitochondria. In apoptotic cells, the mitochondrial membrane potential collapses, and the DePsipher™ reagent cannot accumulate within the mitochondria. In these cells, DePsipher™ remains in the cytoplasm as a green fluorescent monomeric form. Apoptotic cells, showing primarily green fluorescence, are easily differentiated from healthy cells which show red fluorescence. The aggregate red form has absorption/emission maxima of 585/590 nm, and the green monomeric form has absorption/emission maxima of 510/527 nm. Apoptotic and healthy cells can be visualized simultaneously by epifluorescence microscopy using a wide band-pass filter.

Separate microscopic observation of the two dye forms can be performed using independent fluorescein and rhodamine filters, and a composite image created using image analysis software. In flow cytometric experiments, the green monomer can typically be detected using the fluorescein channel and the orange-red aggregates can be detected using the propidium iodide channel. DePsipher™ can be used to evaluate the viability of a cell population, quickly estimate the effect of drugs or other cytotoxins on a cell population, and detect early apoptosis in known models.

II. Precautions and Limitations

1. For Research Use Only. Not for use in diagnostic procedures.
2. The physical, chemical, and toxicological properties of the products contained within the DePsipher Kit may not yet have been fully investigated. Therefore, Trevigen recommends the use of gloves, lab coats, and eye protection while using any of these chemical reagents. Trevigen assumes no liability for damage resulting from handling or contact with these products. MSDS sheets are available.

III. Materials Supplied

<u>Component</u>	<u>Amount Provided</u>	<u>Storage</u>	<u>Catalog #</u>
*DePsipher	100 μ l	-20 °C	6300-100-01
10X Reaction Buffer	2 X 30 ml	4 °C	6300-100-02
Stabilizer Solution	5 ml	4 °C	6300-100-03

(*Trevigen recommends aliquoting this component upon receipt to avoid repeated freezing and thawing, and storing with desiccant.)

IV. Materials/Equipment Required But Not Supplied

Equipment

1. Micropipettors
2. 37 °C, 5% CO₂ incubator
3. Fluorescence microscope equipped with fluorescein long pass filter
4. FACS machine (Optional)

Reagents

1. Cell culture material

Disposables

1. Serological pipets
2. Microscope slides and coverslips
3. Micropipettor tips

V. Assay Protocol

NOTE: Mitochondrial membrane potential is pH sensitive. For optimal results, ensure that all reagents used are buffered between pH 7.0 and 8.0 and analyze rapidly. Cell culture media may be used in place of the reaction buffer provided.

A. Microscopic Observation

a. Staining of Monolayer Cells

1. Dilute the 10X Reaction Buffer to 1X with distilled water and prewarm to 37 °C before use. To increase the stability of the dye for viewing under the microscope, add 20 μ l of Stabilizer Solution for every 1 ml of 1X Reaction Buffer prepared.
2. Add 1 μ l of DePsipher™ solution to 1 ml of 1X Reaction Buffer prepared in step 1 (final concentration 5 μ g/ml). Vortex to homogenize solution.

NOTE: DePsipher™ solution is poorly soluble in aqueous solution. To remove particles, centrifuge the dye solution at 13,000 x g for 1 minute at room temperature and carefully transfer the supernatant without disturbing pelleted debris.

3. Remove media and cover the cells with diluted DePsipher™ solution.
4. Incubate at 37 °C in a 5% CO₂ incubator for 15-20 minutes.
5. Wash the cells with 1 ml of prewarmed 1X Reaction Buffer with Stabilizer Solution.
6. Observe immediately under the microscope using a fluorescein long-pass filter (fluorescein and rhodamine). In healthy cells, the mitochondria will appear red following aggregation of the DePsipher™ within the mitochondria. The red aggregates have a maximal emission at 590 nm. In dying cells

or cells with disrupted potential, the dye will remain in its monomeric form in the cytoplasm and will appear green with a maximal emission at 530 nm.

b. Staining of Suspension Cells

1. Harvest about 1×10^6 cells per sample by centrifugation at 500 x g for 5 minutes at room temperature.
2. Dilute the 10X Reaction Buffer to 1X with distilled water and prewarm at 37 °C before use. To increase the stability of the dye for viewing under the microscope, add 20 μ l of Stabilizer Solution for every 1 ml of 1X Reaction Buffer prepared.
3. Add 1 μ l of DePsipher™ solution to 1 ml of 1X Reaction Buffer prepared in step 1 (final concentration 5 μ g/ml).

NOTE: DePsipher™ solution is poorly soluble in aqueous solution. To remove particles, centrifuge the dye solution at 13,000 x g for 1 minute at room temperature and carefully transfer the supernatant without disturbing pelleted debris.

4. Resuspend cells in 1 ml of diluted DePsipher™ solution.
5. Incubate at 37 °C in a 5% CO₂ incubator for 15-20 minutes.
6. Centrifuge cells at 500 x g and discard supernatant.
7. Resuspend cells in 1 ml of prewarmed 1X Reaction Buffer.
8. Observe immediately under the microscope using a fluorescein long-pass filter (fluorescein and rhodamine). In healthy cells, the mitochondria will appear red following aggregation of DePsipher™ within the mitochondria. The red aggregates have a maximum emission at 590 nm. In dying cells or cells with disrupted potential, the dye will remain in its monomeric form in the cytoplasm and will appear green with a maximum emission at 530 nm.

B. Analysis by Flow Cytometry

NOTE: The Stabilizer Solution is not recommended for analysis by flow cytometry.

1. Harvest about 1×10^6 cells per sample by centrifugation at 500 x g for 5 minutes at room temperature.
2. Dilute the 10X Reaction Buffer to 1X with distilled water and prewarm at 37 °C before use. Add 1 μ l of DePsipher™ solution to 1 ml of 1X prewarmed Reaction Buffer (final concentration 5 μ g/ml). Vortex to homogenize solution.

NOTE: DePsipher™ is poorly soluble in aqueous solution. To remove particles, centrifuge the dye solution at 13,000 x g for 1 minute and carefully transfer the supernatant to a clean tube without disturbing pelleted debris.

3. Resuspend cells in 1 ml of diluted DePsipher™ solution.
4. Incubate samples at 37 °C, 5% CO₂ for 20 minutes.
5. Wash samples 2 times in 1X Reaction Buffer with centrifugation at 500 x g between each wash.
6. Resuspend cells in 1 ml of 1X Reaction Buffer and analyze quickly by flow cytometry (488 nm argon laser).
7. Keep samples shielded from light until analysis. Stained cells can be kept at room temperature or on ice until analyzed.
8. Optimize setting of apparatus and incubation times as necessary.

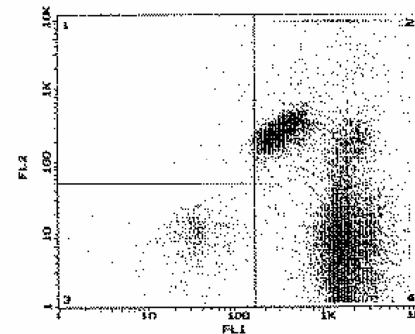


Fig.1. Rat thymocytes stained with DePsipher™, 24 hours after harvesting and analyzed by flow cytometry. The lower left quadrant regroups cells showing only green fluorescence corresponding to those with disrupted mitochondrial potential following apoptosis. The upper right quadrant regroups two cell populations which are exhibiting different intensities of green and red at the same time.

C. Fixation of Samples

The DePsipher™ assay requires viable cells or a positive membrane potential to aggregate, therefore, fixed samples are not suitable for this application. In cases where the fixation of samples is necessary, a light fixation with 0.5% formaldehyde for 5 minutes at room temperature, postassay, has been shown not to change the fluorescence pattern. If material is infectious, appropriate precautions should be taken since a light fixation may be insufficient to inactivate the infectious agent.

VI. Controls

A. Negative Control

Some cell lines may be too sensitive to pH variations to allow the use of the DePsiher™ assay. Conditions may be optimized by comparing results with viable counts of healthy samples (trypan blue dye exclusion). Use of Fetal Bovine Serum (FBS) supplemented media to incubate and wash the cells may improve the results for sensitive cells lines or if extended periods of time are necessary to observe the results.

B. Positive Control

Treatment of cells with certain drugs that disturb the mitochondrial membrane potential provide an appropriate positive control. For example, incubations with valinomycin (100 nM) or carbonyl cyanide p-(trifluoromethoxy) phenylhydrazone (FCCP, 250 nM) results in a radical change in the distribution of the fluorescence. A positive control should be used to give guidelines for the interpretation of cell distribution in flow cytometry analysis.

VII. References

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VIII. Related products available from Trevigen.

Catalog #	Description	Size
6305-100-K	MitoShift™ Mitochondrial Potential Assay Kit	100 tests
4817-60-K	FlowTACS™ Apoptosis Detection Kit	60 samples
4822-96-K	HT TiterTACS™ Assay Kit	96 tests
4830-01-K	TACS™ Annexin V FITC Kit	100 samples
4835-01-K	Annexin V Biotin Kit	100 samples
4815-30-K	TumorTACS™ <i>In Situ</i> Apoptosis Detection Kit	30 samples
4823-30-K	NeuroTACS™ <i>In Situ</i> Apoptosis Detection Kit	30 samples
4827-30-K	CardioTACS™ <i>In Situ</i> Apoptosis Detection Kit	30 samples
4829-30-K	DermaTACS™ <i>In Situ</i> Apoptosis Detection Kit	30 samples
4826-30-K	VasoTACS™ <i>In Situ</i> Apoptosis Detection Kit	30 samples
4828-30-DK	TACS•XL® DAB <i>In Situ</i> Apoptosis Detection Kit	30 samples
4828-30-BK	TACS•XL® Blue Label <i>In Situ</i> Apoptosis Detection Kit	30 samples
4810-30-K	TACS™ 2 TdT DAB <i>In Situ</i> Apoptosis Detection Kit	30 samples
4811-30-K	TACS™ 2 TdT Blue Label <i>In Situ</i> 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 μ l
2341-PC-050	Anti-human/mouse-Bim	50 μ g
2315-PC-050	Anti-human-Bid polyclonal	50 μ g
2316-PC-075	Anti-human-Mcl-1 polyclonal	75 μ g
2317-PC-050	Anti-human-TRAIL polyclonal	50 μ g
2318-PC-075	Anti-human-cIAP-2 polyclonal	75 μ g
2319-PC-050	Anti-human-cIAP-1 polyclonal	50 μ g
2321-PC-100	Anti-human-Drp-1 polyclonal	100 μ g
2323-PC-050	Anti-human/murine-XIAP polyclonal	50 μ g
2340-MC-050	Anti-human-APAF-1 (clone 94408.11)	50 μ g
2342-PC-050	Anti-human-CD95 (Fas) polyclonal	50 μ g
2343-MC-250	Anti-human-CD95 (Fas) mAb (clone Dx2)	250 μ g
2344-MC-250	Anti-human-CD95 (Fas) mAb (clone 50830)	250 μ g
2290-MC-100	Anti-murine-Bcl-2 mAb (clone YTH-10C4)	100 μ g
2291-MC-100	Anti-human-Bcl-2 mAb (clone YTH-8C8)	100 μ g
2300-MC-100	Anti-human-Bcl-XL mAb (clone YTH-2H12)	100 μ g
2330-MC-025	Anti-human-Bax and -Bcl-2 mAbs	25 μ g each
2280-MC-100	Anti-mouse-Bax mAb (clone YTH-5B7)	100 μ g
2281-MC-100	Anti-human-Bax mAb (clone YTH-6A7)	100 μ g
2282-MC-100	Anti-human-Bax mAb (clone YTH-2D2)	100 μ g
2304-PC-040	Anti-human/mouse-Caspase 2 polyclonal	40 μ g
2305-PC-100	Anti-cleaved human/mouse-Caspase 3 polyclonal	40 μ g
2308-MC-050	Anti-human-Caspase 9 mAb	50 μ g
2309-MC-050	Anti-human/mouse-Caspase 10 mAb	50 μ g
2310-PC-050	Anti-human/mouse-Caspase 10 polyclonal	50 μ g
2312-MC-050	Anti-human-Caspase 7 mAb	50 μ g
6360-PC-100	Anti-human/mouse-PBR polyclonal	100 μ l
6370-MC-100	Anti-human/murine-Cytochrome C	100 μ g
6380-MC-100	Anti-human/murine-Holocytochrome C	100 μ g
4335-MC-100	Anti-PAR polymer mAb (10HA)	100 μ l
4336-BPC-100	Anti- PAR polymer polyclonal	100 μ l
4338-MC-50	Anti-human/murine-PARP mAb (clone C2-10)	50 μ g

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.
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 μ l
4870-500-6	10X PBS	6 x 500 ml
4869-500-6	Apoptosis Grade™ H ₂ O	6 x 500 ml
4820-30-13	Blue Counterstain	50 ml
4825-30-01	Red Label™	5 ml
4878-05-02	Cytonin™ IHC	2 x 5 ml

The product accompanying this document is intended for research use only and is not intended for diagnostic purposes or for use in humans.

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