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Stressgen

> DePsipher™

Catalog # 900-167

Kit for Detection of Mitochondrial Membrane Potential Disruption

Reagents require separate storage conditions.

Check our website for additional protocols, technical notes and FAQs

For proper performance, use the insert provided with each individual kit received

Do not mix components from different kit lots or use reagents beyond the expiration date of the kit.

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FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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Introduction

Assay Designs' DePsipher™ Kit is for the detection of mitochondrial membrane potential disruption via fluorescent microscopy or flow cytometry.

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 transmembrane 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.

DePsipher™ is a lipophilic cation (5, 5´, 6, 6´-tetrachloro-1, 1´, 3, 3´-tetraethyl-benzimidazolyl carbocyanin iodide) which can be used as a mitochondrial activity marker. DePsipher has the property of aggregating upon membrane polarization forming an orange-red fluorescent compound. If the potential is disturbed, the dye can not access the transmembrane space and remains or reverts to its green monomeric form. The fluorescence can be observed with a microscope or analyzed by flow cytometry. The red aggregates have absorption/emission maxima of 585/590 nm and the green monomers of 510/527 nm and can be visualized at the same time by microscopy when using a fluorescein long-pass filter. Separate microscopic observation of the two dye forms can be performed using independent fluorescein filters and rhodamine filters. In flow cytometry experiments, the green monomer can typically be detected using the fluorescein channel (FL1) and the red aggregates can be detected using the propidium iodide channel (FL2).

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.

Materials Supplied

1. DePsipher Solution
   100 µL, Product No. 80-1691
   Solution of 5, 5', 6, 6'-tetrachloro-1, 1', 3, 3'-tetraethyl-benzimidaxoly carbocyanin iodide in DMSO.
2. Reaction Buffer Concentrate
   2 x 30 mL, Product No. 80-1692
3. Stabilizer Solution
   0.5 mL, Product No. 80-1693

DePsipher Solution should be aliquoted upon receipt and stored at -20°C.

The physical, chemical, and toxicological properties of these reagents have not been fully investigated. We recommend the use of proper lab safety equipment,

Storage

All components of this kit, except the DePsipher Solution, are stable at 4°C until the kit's expiration date. The DePsipher Solution should be aliquotted upon receipt and stored at -20C. Avoid repeated freeze/thaw cycles.

Materials Needed but Not Supplied

1. 1X PBS
2. Cell culture material
3. Distilled or deionized water
4. Pipet helper
5. Micropipettor
6. CO_2 incubator (37°C)
7. Fluorescence microscope equipped with fluorescein long pass filter and/or Flow Cytometer
8. Serological pipets
9. Microscope slides and coverslips
10. Micropipetor tips

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Assay Procedure

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.

Microscopic Observation

Staining of Monolayer Cells

1. Dilute the 10X Reaction Buffer Concentrate to 1X with distilled or deionized water and pre-warm to 37°C before use. To increase the stability of the dye for viewing with a microscope, add 20 µL of Stabilizer Solution for every 1 mL of 1X Reaction Buffer prepared.
2. Add 1 µL of DePsipher to 1 mL of 1X Reaction Buffer prepared in step 1 (final concentration 5 µg/mL). Vortex to homogenize solution.
   Note: DePsipher is poorly soluble in aqueous solutions. To remove particles, centrifuge the dye solution at 13,000 x g for 1 minute at room temperature and carefully transfer the supernate without disturbing pelleted debris.
3. Remove media and cover the cells with diluted DePsipher solution.
4. Incubate at 37°C in a 5% $C0_2$ incubator for 15 - 30 minutes.
5. Wash the cells with 1 mL of prewarmed 1X Reaction Buffer with Stabilizer.
6. 0bserve immediately with a microscope using a long-pass filter (fluorescein and rhodamine). In healthy cells, the mitochondria will contain red spots following aggregation of the DePsipher within the mitochondria. The red aggregates emit 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 entirely green with an emission at 530 nm.

Staining of Suspension Cells

1. Harvest about 1 x 10^6 cells per sample by centrifugation at 500 x g for 5 minutes at room temperature.
2. Dilute the 10X Reaction Buffer Concentrate to 1X with distilled or deionized water and pre-warm to 37°C before use. To increase the stability of the dye for viewing with a microscope, add 20 µL of Stabilizer Solution for every 1 mL of 1X Reaction Buffer prepared.
3. Add 1 µL of DePsipher to 1 mL of 1X Reaction Buffer prepared in Step 2 (final concentration 5 µg/mL); vortex to homogenize the solution.
   Note: DePsipher is poorly soluble in aqueous solutions. To remove particles, centrifuge the dye solution at 13,000 x g for 1 minute at room temperature and carefully transfer the supernate without disturbing pelleted debris.
4. Resuspend cells in 1 mL of diluted DePsipher solution.
5. Incubate at 37°C in a 5% $C0_2$ incubator for 15 - 30 minutes.
6. Centrifuge cells at 500 x g and discard the supernate.
7. Resuspend cells in 1 mL of pre-warmed 1X Reaction Buffer with Stabilizer.
8. 0bserve immediately with a microscope using a long-pass filter (fluorescein and rhodamine). In healthy cells, the mitochondria will contain red spots following aggregation of the DePsipher within the mitochondria.

The red aggregates emit 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 entirely green with an emission at 530 nm.

Flow Cytometry Assay

Note: The Stabilizer Solution is not recommended for use in flow cytometric analysis.

1. Harvest about 1 x 10^6 cells per sample by centrifugation at 500 x g for 5 minutes at room temperature.
2. Dilute the 10X Reaction Buffer Concentrate to 1X with distilled or deionized water and pre-warm to 37°C before use. Add 1 µL of DePsipher to 1 mL of prewarmed 1X Reaction Buffer (final concentration 5 µg/mL). Vortex to homogenize solution.
   Note: DePsipher is poorly soluble in aqueous solutions. To remove particles, centrifuge the dye solution at 13,000 x g for 1 minute at room temperature and carefully transfer the supernate 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% $C0_2$ for 20 minutes.
5. Wash samples 2 times in PBS with centrifugation at 500 x g between each wash.
6. Keep samples shielded from light until analysis.
7. Resuspend cells in 1 mL of PBS and immediately analyze with a flow cytometer (488 nm argon laser).
8. Optimize the flow cytometry instrument settings and incubation times as necessary.

Fixation of Samples

DePsipher 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 preferred, a light fixation with 0.5% formaldehyde for 5 minutes at room temperature, post-assay, has been shown not to change the fluorescence pattern. If material is infectious, precautions should be taken to ensure that a light fixation is sufficient to inactivate the infectious agent.

Controls

Negative Control

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

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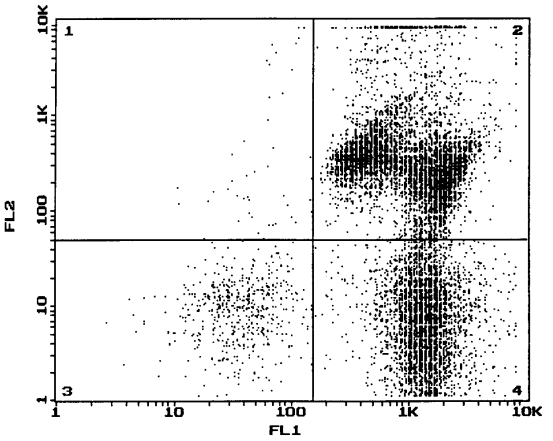
Positive control

Treatment of cells with certain drugs that disturb the mitochondrial membrane potential provides an appropriate positive control. For example, incubation with valinomycin (100 nM) or carbonyl cyanide p-(trifluoro-methoxy) 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 the flow cytometry analysis.

Typical Results



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.

References

1. Barbieri, D., ***et al***. (1998) Neurochem Int. **32**: 493-504.
2. Bobyleva, V., ***et al***. (1998) FEBS Lett. **430**: 409-413.
3. Bowser, D.N., ***et al***. (1998) Biophys J. **75**: 2004-2014.
4. Cossarizza, A., ***et al***. (1993) Biochem Biophys Res Commun. **197**: 40-45.
5. Gu, M., ***et al***. (1998) Ann Neurol. **44**:177-186.
6. Mancini, M., ***et al***. (1998) Ann Surg Oncol. **5**:287-295.
7. Polla, B.S., ***et al***. (1996) Free Radic Res. **25**:125-131.
8. Reers, M., ***et al***. Biochemistry **30**:4480-4486.
9. Salvioli, S., ***et al***. (1998) Biochemistry Mosc. **63**:235-238.
10. Troiano, L., ***et al***. (1998) Exp Cell Res. **24**:384-393.
11. Virag, L., ***et al***. (1998) J Immunol. **161**:3753-3759.

Limited Warranty

Assay Designs, Inc. warrants that at the time of shipment this product is free from defects in materials and workmanship. This warranty is in lieu of any other warranty expressed or implied, including but not limited to, any implied warranty of merchantability or fitness for a particular purpose.

MSDS (Material Safety Data Sheet) available online

Assay Designs must be notified of any breach of this warranty within 48 hours of receipt of the product. No claim shall be honored if Assay Designs is not notified within this time period, or if the product has been stored in any way other than outlined in this publication. The sole and exclusive remedy of the customer for any liability based upon this warranty is limited to the replacement of the product, or refund of the invoice price of the goods.

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Assay Designs has always been an active contributor to a number of local, national and international charities. We have broadened our charitable contributions by implementing a program called Kits for Charity™.

Each quarter, Assay Designs will feature a different non-religious and non-political charitable organization on our website. For each kit sold during this time period, we will make a monetary contribution to the featured charity. Please check our website for the current quarter's charity to see what organization your purchases are helping to support.

If you have any suggestions for future Kits for Charity™ recipients, please contact us at 800.833.8651 or 734.668.6113.

Contact Us

For more details concerning the information within this kit insert, or to order any of the Assay Designs' products, please call (734) 668-6113 between 8:30 a.m. and 5:30 p.m. EST. Orders or technical questions can also be transmitted by fax or e-mail 24 hours a day.

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