DAP Green

Catalog number: 23003
Unit size: 100 Tests

Component	Storage	Amount (Cat No. 23003)
DAP Green	Freeze (< -15 °C), Minimize light exposure	100 Tests

OVERVIEW

DAP Green is a fluorescent dye designed to detect autophagic activity in live cells. Autophagy is the process of cellular degradation to get rid of dysfunctional proteins and damaged organelles. It plays an important role in cellular maintenance, aging, and the development of neurodegenerative diseases like Parkinson's disease. During this process, autophagosomes are formed which encapsulates damaged cellular components. These autophagosomes then fuse with lysosomes to form autolysosomes, where degradation of components occurs.

DAP Green is a cell-permeable molecule, owing to its structural properties, it integrates into autophagosomes during their formation. DAP Green emits green fluorescence, enabling clear visualization of autophagosome formation and autolysosome activity in living cells. It can be detected using standard fluorescence microscopy or quantified through flow cytometry, making it an excellent tool for both qualitative and quantitative autophagy analysis. DAP Green does not require transfection, making it easy to use and ideal for a wide variety of cell types, providing a simple yet effective way to monitor autophagy in real-time, offering valuable insights for basic research and drug screening.

AT A GLANCE

Protocol Summary

1. Prepare cells in a growth medium.
2. Incubate the cells with the DAP Green working solution at 37°C for 30 minutes.
3. Starve the cells to induce autophagy.
4. Observe under a fluorescence microscope using a GFP filter set.

KEY PARAMETERS

Fluorescence microscope

Emission	FITC Filter Set
Excitation	FITC Filter Set
Recommended plate	Black wall/clear bottom

CELL PREPARATION

Adherent Cells

1. Plate cells overnight in growth medium at 10,000–40,000 cells per well in 100 µL for a 96-well plate or 2,500–10,000 cells per well in 25 µL for a 384-well plate.

Non-adherent Cells

1. Centrifuge cells in culture medium to pellet, then resuspend in fresh culture medium.
2. Seed 50,000–100,000 cells/well in 100 µL for a 96-well poly-D-lysine plate or 10,000–25,000 cells/well in 25 µL for a 384-well poly-D-lysine plate.
3. Before the experiment, centrifuge the plate at 800 rpm for 2 minutes with the brake off.

Note: The optimal cell density should be determined individually for each cell line.

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles

1. Prepare stock solution by adding 10 µL of DMSO into the DAP Green vial.

PREPARATION OF WORKING SOLUTION

1. Prepare working solution by adding 8 µL of DAP Green stock solution into 10 mL of complete media. Protect the working solution from light by covering it with foil or placing it in the dark.

   Note: For best results, this solution should be used within a few hours of its preparation.

   Note: 10 mL of working solution is enough for 100 tests.

SAMPLE EXPERIMENTAL PROTOCOL

1. Seed adherent cells overnight or prepare suspension cells as described in the CELL PREPARATION section.
2. Add 100 µL of working dye solution to each well of 96 well plate containing cells.
3. Incubate for 30 minutes at 37ºC.
4. Wash the cells twice with complete media.
5. Starve the cells in no serum media for 2 hours to overnight to induce autophagy. Use appropriate controls.
6. Counterstain with nuclear stain for better visualization.
7. Image cells under GFP filter set.

EXAMPLE DATA ANALYSIS AND FIGURES

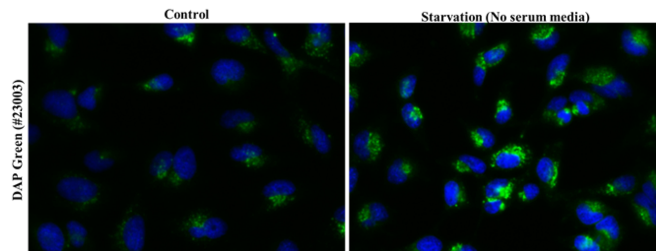

Figure 1. HeLa cells were stained with DAP Green in complete media, followed by serum starvation to induce autophagy. Cells were then counter-stained for nuclear staining using Hoechst 33342 (#22657).

DISCLAIMER

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