Protocol

Agilent
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Agilent eTox Green

Recommended use: Agilent eTox Green for detection of cytotoxicity

For Research Use Only. Not for use in diagnostic procedures.

Product information

Catalog number: 8711008

Size and concentration: 10 µL/vial at 1 mM in DMSO (4 pk)

Each vial provides sufficient quantity for 200 tests
(1 test: 1 well of a 96-well microplate).

Storage conditions

Upon receipt, store at –20 °C and protected from light.

Allow all solutions to warm up to room temperature and mix thoroughly before use. Long-term storage of working solutions in 100% aqueous media should be avoided.

Description

The Agilent eTox Green reagent selectively stains the nuclei of dead cells. It is incapable of passing through the intact membrane of healthy cells but gains access to the interior of dead cells through disruptions in their membranes. Once inside a dead cell, the eTox dyes bind to DNA and become 100 to 1,000-fold more fluorescent. This enables real-time quantification of cell death in response to pharmacological agents or genetic and environmental factors.

Agilent eTox reagents can be combined with other Agilent fluorescent live imaging reagents and dyes for multiplexed measurements, including cell viability, apoptosis, and cytotoxicity in a single well.

All Agilent live cell imaging reagents have been validated for use with the Agilent xCELLigence RTCA eSight live-cell analysis imaging and impedance system.

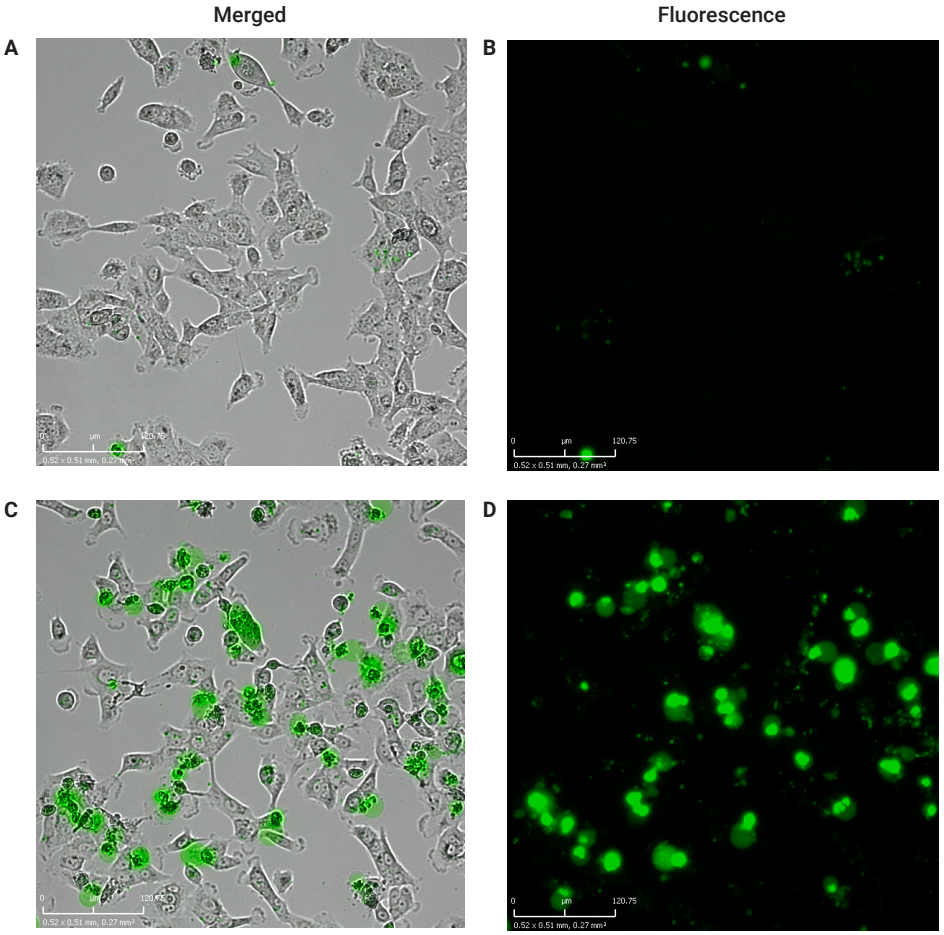

Figure 1. eTox Green-labeled cells. Representative images of A549 cells at 4 (A and B) and 24 hours (C and D) post treatment with 1.65 µM Camptothecin in the presence of 250 nM eTox Green.

Spectral properties

eTox Green: Ex/Em: 491/508 nm

Precautions

See the Safety Data Sheet

Recommended protocol

Required materials

- Agilent eTox Green (1 mM stock)
- Cells and appropriate culturing medium. In this example, the A549 cells were grown in F12K medium containing 10 % FBS, 1% penicillin/streptomycin.
- Cytotoxic compound
- Agilent xCELLigence RTCA eSight instrument
- Agilent E-Plate VIEW 96

Procedure

1. Add 50 µL of cell culture medium to each well of an E-Plate VIEW 96.
2. Place the plate in the xCELLigence RTCA eSight instrument inside the incubator and take a background impedance reading.
3. Add the appropriate number of cells in 100 µL to each well.
   Example: For A549 cells, add 6,000 cells/100 µL. This will give a final seeding density of 6,000 cells/well.
4. Allow cells to settle in the plate for 30 minutes at room temperature.
5. Place the plate in the RTCA eSight instrument and monitor cell adhesion and proliferation for 24 hours by measuring impedance every 15 minutes. If desired, brightfield images can also be acquired every two hours.

Addition of eTox dye and cytotoxic compound

6. To prepare 2x working mixture, add eTox dye to the growth medium (to achieve a concentration of 500 nM) and cytotoxic compound (to achieve a concentration that is 2x the desired final concentration). You will need 100 µL of this mixture per well.
7. At 24 hours postseeding, remove 50 µL of media from the wells, leaving behind 100 µL. Then add 100 µL of the eTox dye and cytotox compound 2x working mixture to each well. This generates the suggested final working concentration of eTox dye (250 nM) and a final volume of 200 µL/well.

Monitoring cytotoxicity

8. Place the plate in the RTCA eSight instrument and begin data acquisition for 48 hours at the desired temporal frequency. The recommended time between scans is 15 minutes for impedance and 2 hours for imaging. Although the exposure duration for fluorescent image acquisition may need to be optimized for each cell line, a good starting point for eTox Green is 300 ms in the green channel of the Agilent xCELLigence RTCA eSight.

Note: The solubility of the eTox dyes vary depending on the composition of the growth medium. In some media, the eTox dyes may form a small number of insoluble aggregates along the well bottom. This is most common with eTox Green. The presence of these dye aggregates will increase the background signal but does not impact the ability to detect/visualize the emergence of dead cells over the course of the assay. In some contexts, the user may want to subtract this background signal (in the absence of treatment) from the signal of treated samples.

Related products

Product	Part Number
Apoptosis	
eAnnexin V Green	8711006
eAnnexin V Red	8711007
eAnnexin V Blue	8711026
Cytotoxicity/viability	
eTox Green	8711008
eTox Red	8711009
Lentiviruses	
eLenti Green	8711010
eLenti Red	8711011
eLenti Blue	8711012
Agilent RTCA instrument	
xCELLigence RTCA eSight bundle	380601600
Agilent E-Plates	
E-Plate VIEW 96 (6 plates)	300601020
E-Plate VIEW 96 (36 plates)	300601030

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