Protocol

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Agilent eCaspase-3 Green in PBS

Recommended use: Agilent eCaspase Green reagent for detection of apoptosis

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

Product information

Part number: 8711005

Size and concentration: 100 µL/vial at 1mM in 1x PBS

Each vial provides sufficient quantity for 100 tests

(1 test: 1 well of a 96-well microplate).

Storage conditions

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

Description

The Agilent eCaspase-3 Green reagent detects caspase-3/7 activity within intact cells without inhibiting apoptosis progression. It consists of a fluorogenic DNA dye that is covalently linked to a peptide which contains the caspase-3/7 DEVD recognition sequence.

In this initial state the substrate is not capable of binding to DNA and is non-fluorescent. Within apoptotic cells the peptide is cleaved by caspase-3/7, releasing the dye moiety which then binds to DNA with high affinity and causes nuclei to fluoresce green. Thus, the eCaspase-3 reagents are bi-functional, allowing detection of intracellular caspase-3/7 activity and visualization of changes in nuclear morphology during apoptosis. The eCaspase-3 Green 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 real-time cell analysis (RTCA) eSight live-cell analysis imaging and impedance system.

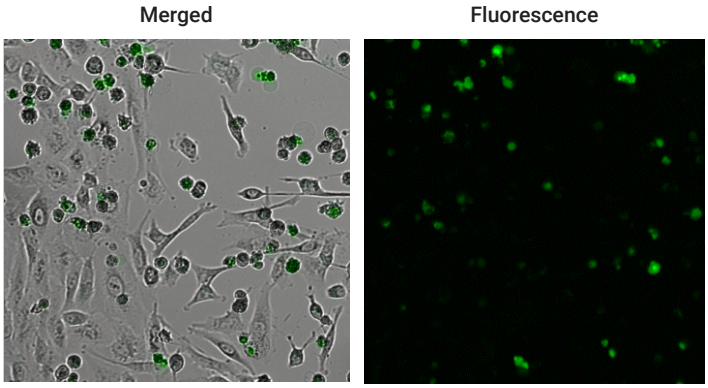

Figure 1. Representative images of A549 cells grown in the presence of eCaspase-3 Green (5 µM). Images are 20 hours after treatment with the apoptosis inducer MG132 (25 µM).

Spectral properties

eCaspase-3 Green: Ex/Em: 500/530 nm

Precautions

See the Safety Data Sheet

Recommended protocol

Required materials

- eCaspase-3 Green in PBS (1 mM stock)
- Apoptosis inducing reagent
- xCELLigence RTCA eSight instrument
- 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 RTCA eSight instrument inside the incubator and take a background impedance reading.
3. To each well add the appropriate number of cells in 100 µL.
   Example: For HT-1080 cells, add 6,000 cells/100 µL. This will give a final seeding of 6,000 cells/well in 150 µL.
4. Allow the 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, measuring impedance every 15 minutes. If desired, brightfield images may also be acquired every 2 hours.

Addition of eCaspase-3 substrate and apoptosis inducer

6. **Prepare 2x working mixture:** To growth medium, add eCaspase-3 (to achieve a concentration of 10 µM) and apoptosis inducer (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 post seeding, remove 50 µL media from the wells, leaving behind 100 µL. Then add 100 µL of the eCaspase-3/apoptosis inducer 2x working mixture to each well. This generates the suggested final working concentration of eCaspase-3 (5 µM) and a final volume of 200 µL/well.
8. Place the plate in the RTCA eSight instrument and initiate data acquisition at the desired temporal frequency. (The recommended time between scans is 15 minutes for impedance and 2 hours for imaging.) Although the exposure length for fluorescent image acquisition may need to be optimized for each cell line, a good starting point for eCaspase-3 Green is 300 ms in the green channel of the Agilent xCELLigence RTCA eSight.

Related products

Product	Part Number
Live Cell/Proliferation	
eLive Green	8711003
eLive Red	8711004
Verapamil (eLive Enhancer)	8711038
Apoptosis	
eCaspase-3 Green in PBS	8711005
eCaspase-3 Blue in PBS	8711027
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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