Protocol

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Agilent eAnnexin V Green

Recommended use: Agilent eAnnexin V Green for detection of apoptosis

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

Product information

Catalog number: 8711006

Size: 5 µg/vial lyophilized

Each vial provides sufficient quantity for 100 tests
(1 test: 1 well of a 96-well microplate).

Storage conditions

Upon receipt, store at 4 °C and protect from light.

Description

The Agilent eAnnexin V Green (CF488A) reagent enables quantitative detection of apoptotic cells based on phosphatidylserine (PS) translocation. Annexin V is a calcium-dependent phospholipid-binding protein with high affinity for PS. In normal viable cells, PS is located on the inner surface of the cytoplasmic membrane. Once cells become apoptotic, PS may be translocated to the extracellular surface of the cell membrane, where it can be detected by the fluorophore-conjugated eAnnexin V reagents.

The eAnnexin V Green is supplied as a lyophilized solid that lacks azide or other preservatives. After reconstitution, it can be added to the cell culture medium for live cell imaging. The eAnnexin V 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 real-time cell analysis (RTCA) eSight live-cell analysis imaging and impedance system.

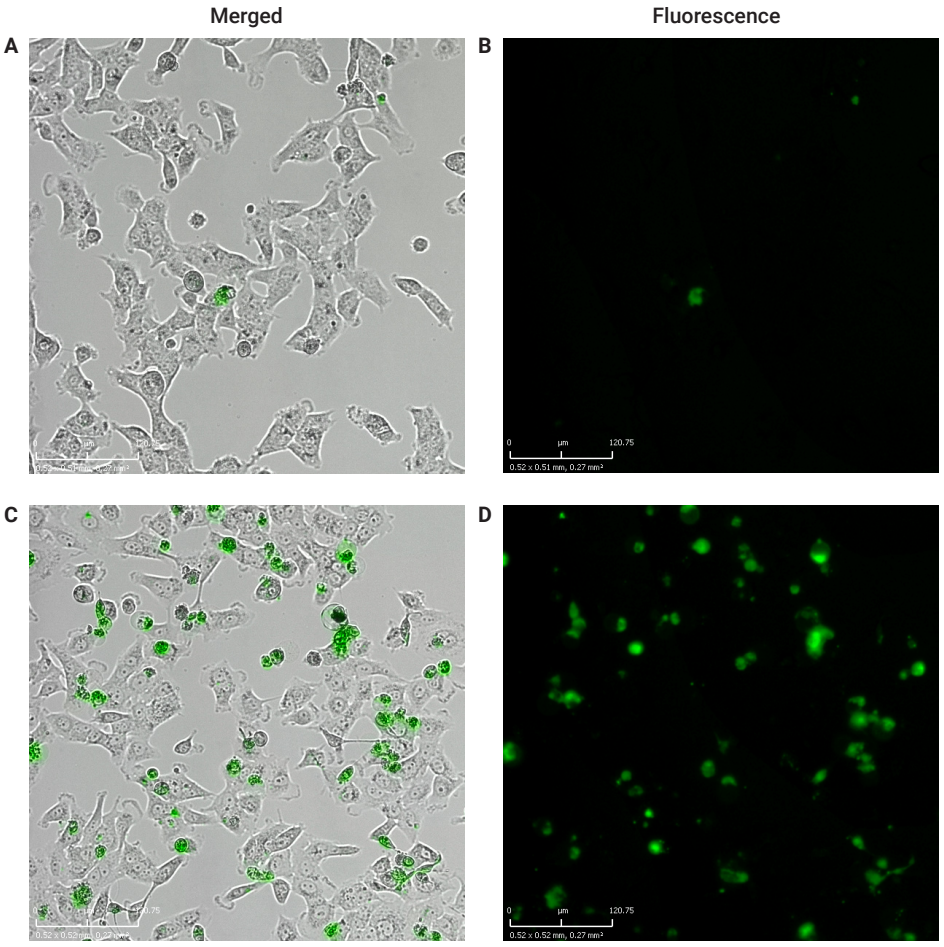

Figure 1. eAnnexin V Green-labeled cells. Representative images of A549 cells at 4 (A and B) and 24 hours (C and D) post treatment with 1.65 µM Camptothecin in the presence of 0.25 µg/mL eAnnexin V Green.

Spectral properties

eAnnexin V Green: Ex/Em: 490/515 nm

Precautions

See the Safety Data Sheet

Recommended protocol

Required materials

- eAnnexin V Green (5 µg lyophilized)
- Cells and appropriate culturing medium. In this example, the A549 cells were grown in F12K medium containing 10 % FBS, 1% penicillin/streptomycin.
- Apoptosis inducing reagent
- 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 an 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, bright-field images can also be acquired every two hours.

Addition of eAnnexin V dye and apoptosis inducer

6. Dissolve the entire vial of 5 µg lyophilized eAnnexin V Green reagent by adding 100 µL of cell culture medium to create a 50 µg/mL stock. Gently pipet the mixture up and down a few times to ensure that the powder is completely dissolved. Do not vortex (to avoid protein denaturation).
7. To prepare 2x working mixture, add eAnnexin V to the growth medium (to achieve a concentration of 0.5 µg/mL) and apoptosis inducer (to achieve a concentration that is 2x the desired final concentration). You will need 100 µL of this mixture per well.
8. At 24 hours postseeding, remove 50 µL media from the wells, leaving behind 100 µL. Then add 100 µL of the eAnnexin V/apoptosis inducer 2x working mixture to each well. This generates the suggested final working concentration of eAnnexin V (0.25 µg/mL) and a final volume of 200 µL/well.
9. 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 eAnnexin V Green is 300 ms in the green channel of the Agilent xCELLigence RTCA eSight.

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
RTCA Instrument	
xCELLigence RTCA eSight bundle	380601600
E-Plates	
E-Plate VIEW 96 (6 plates)	300601020
E-Plate VIEW 96 (36 plates)	300601030

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