Protocol

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Agilent eLenti Red

Recommended use: Agilent Lentivirus reagent for nuclear labeling of live cells

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

Product Information

Catalog number: 8711011

Size and concentration: 25 µL/vial at >1 × 10^6 IFU/mL*

* The functional titer as IFU/mL can be measured by transducing HT1080 cells and detecting the positively labeled cells by flow cytometry.

Storage conditions

Upon receipt, store at –80 °C.

After the first thaw, additional freezing and thawing may result in decreased viral titer and transduction efficiency.

Description

Agilent eLenti virus reagents enable the construction of stable cell lines that express nuclear-localized mKate2 (red fluorescent protein). Constitutive expression of these fluorescent proteins is driven by the EF1-α promoter. The eLenti reagents are compatible with standard transduction protocols, and transductants are selectable based on puromycin resistance.

The eLenti 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.

Spectral properties

eLenti Red: Ex/Em: 588/633 nm

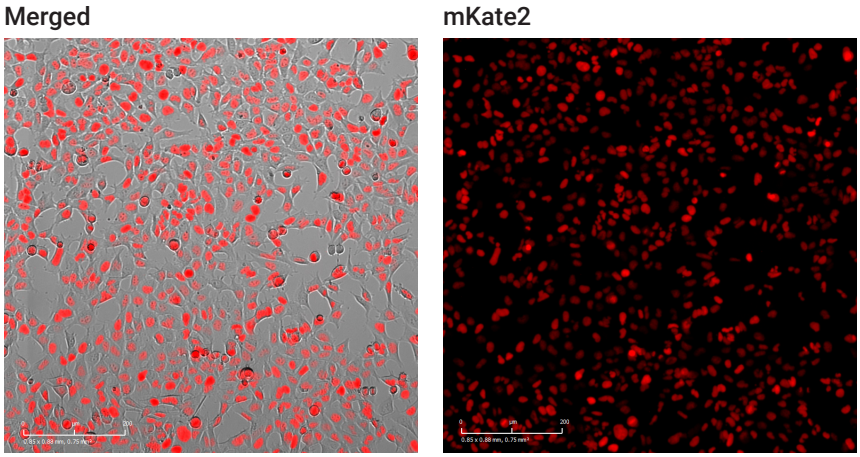

Figure 1. Representative images of HT1080 cells transduced with eLenti Red. Red florescent protein (mKate 2) expression is localized to the nucleus.

Precautions

See the Safety Data Sheet

Recommended protocol to establish stable cell lines

Required materials

- eLenti Red
- Cells and appropriate growth medium. In this example, the HT1080 cells were grown in EMEM medium containing 10% FBS and 1% penicillin/streptomycin.
- Tissue culture 24-well plate
- 10 mg/mL polybrene (Sigma-Aldrich, TR-1003-G)
- Trypsin
- Agilent xCELLigence RTCA eSight instrument
- Agilent E-Plate VIEW 96

Cell seeding

1. On day 1, seed 5×10^4 HT1080 cells in a volume of 1 mL to each well of a 24-well plate. Incubate 18 to 20 hours at 37 °C in a humidified incubator with 5% CO_2.

Addition of lentivirus

2. On day 2, remove the media, and add 0.5 mL of culture medium containing 8 µg/mL of polybrene to each well of the 24-well plate.
3. Infect the cells by adding 2 µL of eLenti to each well of the 24-well plate. Incubate the cells for 4 hours at 37 °C in a humidified incubator with 5% CO_2.
4. Add 1.0 mL of culture medium containing 8 µg/mL of polybrene in each well. This gives a final volume of 1.5 mL/well.

Changing medium

5. At 24 hours postinfection, replace the medium in each well with 1 mL of fresh culture medium.

Puromycin selection

6. At 48 hours postinfection, add puromycin to each of the well to achieve a final concentration of 1 µg/mL.
7. Maintain the cells in puromycin for 10 days to generate a stable cell population expressing a nuclear restricted mKate2, splitting and expanding the cells when needed.

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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