HeLa-EGFRvIII cells were generated from the human cervical cancer cell line HeLa by transfection with an expression vector encoding EGFRvIII with a C-terminal FLAG tag, followed by selection in medium containing geneticin. Expression of EGFRvIII was confirmed by flow cytometry using cells that were fixed and permeabilized (Figure 1).

Storage

Store vial in liquid nitrogen immediately upon receipt.

Formulation

Cells are cryopreserved in 0.5 ml - 1 ml of 90% FBS + 10% DMSO.

Thaw Protocol

Partially immerse the vial in a 37°C water bath with gentle shaking until most of the medium is thawed. In a tissue culture hood, add 1 ml of pre-warmed culture medium into the vial and immediately transfer the contents of the vial to a centrifuge tube containing 5-10 ml of pre-warmed culture medium. Centrifuge the tube at room temperature for 5 minutes, aspirate the supernatant and suspend the cell pellet in 5-10 ml of pre-warmed culture medium.

Culture Protocol

HeLa-EGFRvIII is an adherent cell line. Culture the cells in DMEM containing 10% FBS and 1 mg/ml geneticin using a humidified incubator set to 5% CO2. When the cell monolayer is nearly confluent, use trypsin-EDTA to detach the cells from the culture flask and immediately neutralize the trypsin by adding fresh, pre-warmed culture medium to the cells. Cells should be passaged at least twice per week.

Notices & Disclaimer

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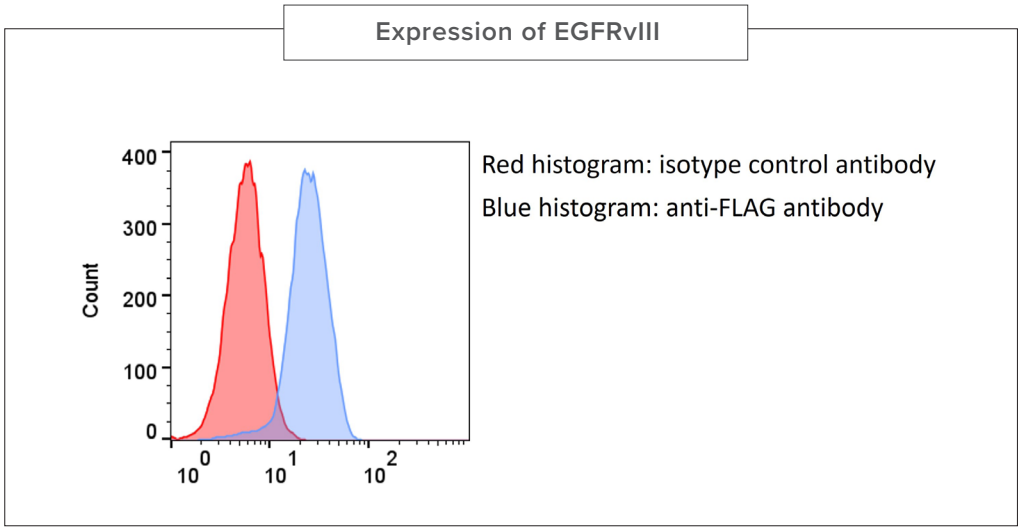

Figure 1. PM-EGFRvIII-HELA cells were fixed, permeabilized and stained with an anti-FLAG antibody and an isotype control antibody. The FLAG antibody bound only to the PM-EGFRvIII-HELA cells.

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