Technote

EGF remains stable and bioactive in conditioned media



[EGF] ng/ml

Quantitative luciferase reporter assay shows that human EGF remains highly bioactive from day 0 to day 2 in conditioned media (CM) with an EC50 of 0.14 pg/ml and 0.10 pg/ml respectively (22.3 pM and 16.6 pM).

Introduction:

Human epidermal growth factor (EGF) is an essential growth factor for stimulating the proliferation of induced pluripotent and embryonic stem cells (iPSC and ESC) and their subsequent differentiation. Hence, EGF is also a key component of many media for developing and maintaining organoids. In stem cell cultures, the instability of growth factors like EGF can contribute to frequent media changes to avoid spontaneous differentiation and variability in the cell cultures. The half-life of EGF in conditioned media is believed to be between 1-2 days but it is currently unknown. Qkine recombinant human EGF protein has been extensively tested to ensure its stability in conditioned media.

Method:

The bioactivity of EGF was determined using the Promega serum response element luciferase reporter assay in HEK293T cells. Cells were treated in triplicate with a serial dilution of EGF for 3 hours in conditioned media. Firefly luciferase activity was measured at days 0 and 2 and normalized to the control Renilla luciferase activity.

Results and conclusion:

The bioactivity assay showed that EGF has high bioactivity at day 0 and maintained stable bioactivity after 2 days in conditioned media. The stability of EGF in conditioned media provides a reliable source of animal-free EGF. This has the potential to improve the reproducibility in self-renewal and differentiation for iPSC, ESC, and organoid cultures.

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