

Reporter assay

Cell lines: HaCaT (human keratinocyte cell line) NLuc reporter stable line

Culture medium: DMEM+10%FBS

Day 1

Plate HaCaT NLuc reporter cells in a white 96 well plate (10000cells/well/100 or 200 μ l, 37°C, O/N)

Day 2

Remove medium from all wells

Add 100 or 200 μ l drug/positive or negative controls-containing medium

37°C, 24h or 48h

Day 3 or 4

Add 3 or 6 μ l WST1

37°C, >1h

Move culture sup to a clear 96 well plate → Measure A490 as 670nm reference (viability)

Add 10 μ l NLuc substrate to the rest (white well) → Measure luminescence (reporter expression)

NLuc substrate (Promega, N1110 or N1120, use accordingly to the manual)

WST1 (Takara bio, MK400)

White 96 well plate (Nunc, 136102)

Positive controls for ARE reporter assay (a.k.a. KeratinoSens™):

EGDMA (CAS 97-90-5, TCI, E0102, use @1~1000 μ M)

CA (CAS 104-55-2, Nacalai, 09024-72, use @1~1000 μ M)

Positive controls for XRE reporter assay:

FICZ (CAS 172922-91-7, ChemSens, CS3513, use @1~1000nM)

I3C (CAS CAS 700-06-1, LKTLab, I5213, use @1~400 μ M)

See OECD 442D for the definition of the test positivity and calculation

<https://doi.org/10.1787/9789264229822-en>