Attachment 6. Training experiment

Basic experiment for transferability to ensure optimal luminescence measurements in the KeratinoSens assay

Three parameters are critical to facilitate reliable results:

a) Sufficient sensitivity giving a stable background in control wells
b) No gradient over the plate due to long reading times
c) No light contamination in adjacent wells from strongly active wells

As a first experiment for method transfer, the set-up of the plate below needs therefore to be tested. (triplicate analysis according to the SOP)

An analysis then needs to be made to ensure:

a) Clear dose response in row D, with the Imax > 20-fold above background, in most cases Imax values between 100 and 300 are reached
b) No dose-response in row C and E (no induction value above 1.3) (-> i.e. **no light contamination** esp. next to strongly active wells in the EGDMA row)
c) No statistically significant difference between the rows A, B, C, E, F and G. (i.e. **no gradient** over plate)
d) Variability in any of the rows A, B, C, E, F and G and in the DMSO wells in row H below 20% (i.e. **stable background**)

EGDMA = Ethyleneglycoldimethacrylate, CAS 97-90-5, a strongly inducing compound
CA = Cinnamic aldehyde, positive reference, CAS 104-55-2

Plate setup of first training experiment

DMSO	DMSO	DMSO	DMSO	DMSO	DMSO	DMSO	DMSO	DMSO	DMSO	DMSO	DMSO
DMSO	DMSO	DMSO	DMSO	DMSO	DMSO	DMSO	DMSO	DMSO	DMSO	DMSO	DMSO
DMSO	DMSO	DMSO	DMSO	DMSO	DMSO	DMSO	DMSO	DMSO	DMSO	DMSO	DMSO
EGDMA 0.98	EGDMA 1.95	EGDMA 3.9	EGDMA 7.8	EGDMA 15.6	EGDMA 31.25	EGDMA 62.5	EGDMA 125	EGDMA 250	EGDMA 500	EGDMA 1000	EGDMA 2000
DMSO	DMSO	DMSO	DMSO	DMSO	DMSO	DMSO	DMSO	DMSO	DMSO	DMSO	DMSO
DMSO	DMSO	DMSO	DMSO	DMSO	DMSO	DMSO	DMSO	DMSO	DMSO	DMSO	DMSO
DMSO	DMSO	DMSO	DMSO	DMSO	DMSO	DMSO	DMSO	DMSO	DMSO	DMSO	DMSO
DMSO	DMSO	DMSO	DMSO	DMSO	DMSO	CA 4	CA 8	CA 16	CA 32	CA64	Blank