DNA QUANTIFICATION KIT-HOECHST ASSAY

KC04003-500 Tests

DESCRIPTION AND USE

DNA is a reliable endpoint for cell proliferation assays. This proliferation assay is based on the enhanced fluorescence and shift in the emission wavelength of the fluorochrome Hoechst 33258 upon binding cellular DNA.

At time points of interest, the plates are emptied of media and stored frozen. When the assay is to be performed, cultures are briefly incubated in a lysate media and frozen again. This process lyses the cells and allows rapid and thorough mixing of the fluorochrome and cellular DNA. Freezing permits convenient storage of cultures until the time of assay, so experiments can be batched. This fact results in a reduction of the processing time and gives a better intra and inter-experimental standardization.

The assay here presents, generates a linear standard curve for DNA fluorescence versus cell number. This enables the rapid and accurate measurement of cell number involving minimal processing time, making this assay suited for cell proliferation studies.

MATERIALS SUPPLIED

Item	Quantity	Storage
Reagent A	1	RT
Reagent B	1	RT
Reagent C	1	4 °C

STORAGE AND STABILITY

On receipt store kit components as indicated above. Do not use after the expiration date stated on the packaging.

REAGENT PREPARATION

Prepare **DNA marker solution** freshly. For this purpose, mix Reagent C and Reagent B in a 1:50 ratio. Example: For 50 wells (5 mL of DNA marker solution): Mix 0.1 mL of Reagent C with 4.9 mL of Reagent B.

ASSAY PROTOCOL

1	Culture cells in a 96-well plate and treat them with the desired treatment
2	Once the treatment has finished, remove culture media by overturning the plate on absorbent toweling.
3	Store then the plate frozen until the day of assay.
4	On the day of assay, add **100 µL** of **Reagent A** to each well and incubate the plate during 1 hour at 37 ºC.
5	Freeze the plate at least 1 hour and then, thaw it until reaching room temperature. Then add **100 µL** of the **DNA marker solution** to each well
6	Cover the plate and shake for 5 minutes at room temperature.
7	Measure the fluorescence of the plate: $\lambda_{excitation}$: 350 nm, $\lambda_{emission}$: 460 nm.
8	Compare the intensity of fluorescence of the control group, with the intensity of fluorescence of the treated groups

RELATED PRODUCTS

Product	Reference
MTT-A Cell Proliferation Assay	KF03001A
Resazurin Based Cell Viability Assay	KC04002
MTT-B Cell Proliferation Assay	KF03001B

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