BQCell Proliferation Assay Kit

KC-04-003

500 tests (96 well plate)

BQCkit

A brand of BioQuChem

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All chemicals should be handled with care

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- This kit is for R&D use only

Introduction

Tissue culture experiments can be limited by small quantities of cells making microcultures desirable, as in work with primary cell cultures. Large numbers of test substances, automation, cost efficiency, and statistical analysis can make 96-well microculture plates attractive. An optimal assay for microculture cell quantification would permit the storage of plates so a large number of plates could be assayed at the same time, thus reducing labor. This would also result in better standardization of data within and between experiments. DNA would be a preferable endpoint of cell proliferation and if possible should be measured without radiolabeled reagents. This optimal assay would require minimal processing time and be highly accurate as a measure of cell proliferation

Materials

BQCkit Cell Proliferation Assay kit *KC-04-003 500 tests* contains:

Product	Quantity	Storage
Reagent A	1 bottle	RT
Reagent B	1 bottle	RT
Reagent C*	1 vial	4ºC

*This reagent is stable during 10 days at Room Temperature and is shipped in these conditions. Once received is recommended to keep it at 4ºC.

Assay Principle

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.

Pre-Assay

1. Prepare DNA marker solution freshly. For this purpose, mix Reagent C and Reagent B in a proportion 1:50.

   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

Performing the assay

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 37ºC.

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 (prepared in advance according to the steps in page number 4). Cover the plate and shake for 5 minutes at room temperature.

Assay Protocol

6. Measure the fluorescence of the plate:
   ʎ excitation: 350 nm
   ʎ emission: 460 nm

7. Compare the intensity of fluorescence of the control group, with the intensity of fluorescence of the treated groups.

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Expiration date: 1 year from the date of delivery

For further details, please refer to our website www.bqckit.com.