Application Notes

Cell Proliferation Assay by Using MicroBeta ^{3}H-Thymidine Incorporation

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Cell Proliferation Assay by Using ^{3}H-Thymidine Incorporation

Type of Assay: Thymidine incorporation measured from filters after harvesting

Label Used: ^{3}H-Thymidine, 0.1 µCi/100 µl

Support: 1450-421 glass filters fibres for harvesting

Measurement Parameters:

Counting Time: 3 minutes

Scintillator: 1450-441 MeltiLex

Introduction

A very common MicroBeta application is the cell proliferation assay performed with a method called thymidine incorporation. This method can be applied to immunological studies related to immunodeficiency, autoimmunity or infectious disease, where the lymphocyte proliferation activity varies from natural levels. Pharmaceutical studies on mentioned topics and on growth factors and cytokines are also possible. A clinical application is to study mixed lymphocyte cultures (MLC) to evaluate immunocompetence of patients in selection of suitable organ donors or recipients.

In a typical thymidine incorporation assay the lymphocytes are cultured a couple of days in the presence of a suitable stimulating agent (growth factor) and tritiated thymidine (^{3}H-Thy). When the cells proliferate, ^{3}H-Thy incorporates into the new DNA that is synthesized. In earlier methods the cell material was separated from free label with TCA-precipitation. This allowed good washing of free ^{3}H-Thy, followed by solubilization with 1 M NaOH and neutralization with HCl. The sample was then transferred into a scintillation vial for measurement. MicroBeta makes it possible to use 96-format glass-fiber filtermats and a cell harvester. In the harvesting step the cell material and especially the DNA is captured into the filtermat, using water as wash buffer. Adherent cells require a trypsin treatment to loosen the cells before the harvesting step. The filtermat is dried and scintillation cocktail or MeltiLex is applied before counting in the MicroBeta.

Procedure

S115 mouse breast cancer cells were plated in Nunc tissue culture 96-well plates ($5x10^3$ /well). The cells were grown for 3 days in DMEM, 4% iFCS with or without 10^{-9}M testosterone and then transferred to serum-free DMEM:F12 containing medium containing 0.1% BSA with or without 10^{-9}M testosterone and/or 3 and 10 ng/ml bFGF (basic fibroblast growth factor) and cultivated in this medium for 2 more days. The cells were labeled with ^{3}H-thymidine for 2.5 h. After labelling, the cells were washed twice with PBS and A) precipitated with 200 µl of TCA for 30' at RT. TCA was aspirated and the precipitate was lysed in 50 µl of 1 M NaOH for another 30' and neutralized with 40 µl of 1 M HCl, or B) trypsinized with 100 µl of 0.2% trypsin in versene. The TCA precipitated, lysed cells (A) or trypsinized cells (B) were harvested onto glass fiber filters using an in-house harvester and the filters were counted in a MicroBeta.

Results

Testosterone increased the rate of proliferation of S115 cells as shown before (Härkönen et al., 1991) (Fig. 1). bFGF also increased the rate of ^{3}H-thymidine incorporation into DNA when added to the cells grown in the absence of testosterone, but in the presence of testosterone no additive effects were observed. The concentrations of 3 and 10 ng/ml of bFGF were equally effective (Fig. 1).

The result supports a previous report by Tanaka et al., (1992) which suggested that testosterone induces the synthesis of a FGF-like factor which mediates the stimulatory effects of the hormone on cell proliferation by autocrine and paracrine mechanisms. Similar results were obtained when two methods of ^{3}H-thymidine incorporation assay were compared. The yield of counts was, however, lower when using the method based on harvesting TCA-precipitated ^{3}H-thymidine labeled DNA onto filters than that based on harvesting trypsinized cells onto the filters.

Figure 1 *Effect of bFGF on (^{3}H) thymidine incorporation by S115 cells grown with or without testosterone*

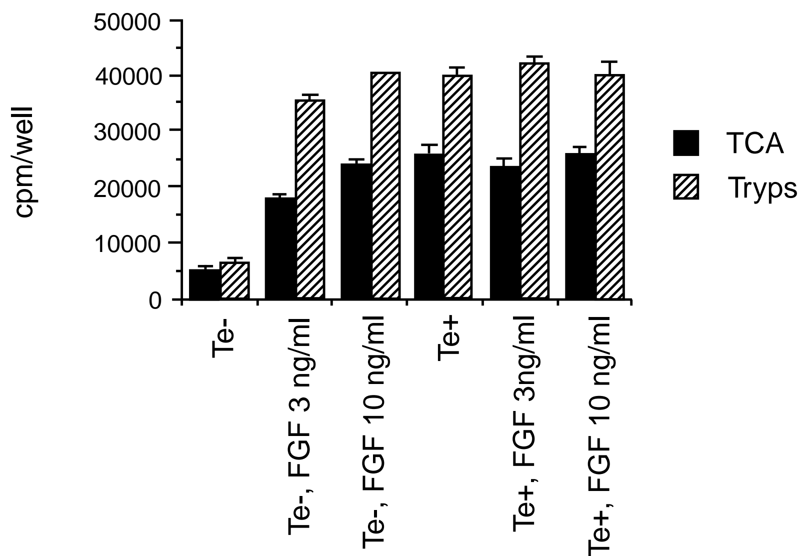

Figure 1: *S115 Cells were grown, labeled with ^{3}H-thymidine and processed by a protocol A (TCA) or B (Tryps) for assay of ^{3}H-thymidine incorporation into DNA as explained in "Procedure". The columns are the means±SD of eight wells. Two separate experiments yielded similar results. bFGF, basic fibroblast growth factor.*

Conclusions

1) The use of 96-well tissue culture plates for proliferation assay makes handling of several parallel samples possible and minimizes the volumes of growth media containing expensive growth factors.

2) Harvesting of trypsinized cells directly onto DNA binding filters makes the assay short and simple. The results obtained are comparable to those produced by using a more conventional TCA precipitation method of labeled cells, followed by NaOH hydrolysis and HCl neutralization prior to harvesting. However, the cpm values are consistently higher in the former method than in the latter method.

Wallac Brand Instruments:

MicroBeta

1450-021	6 Detector
1450-022	3 Detector
1450-023	2 Detector
1450-024	1 Detector
1450-025	6 Detector
1450-027	6 Detector
1450-028	12 Detector
1450-029	12 Detector
1450-030	12 Detector

NEN Brand Reagents:

NET027A, X, Z (^{3}H Thymidine)

Related products:

- 1495-027 Microsealer
- 1450-421 Filtermat A
- 1205-440 BetaPlate Scint scintillation cocktail (1x5L)
- 1450-441 MeltiLex A
- 1450-432 Sample bag

Reference

Härkönen P, Laaksonen E, Valve E, Solic N, Darbre P (1991) Exptl Cell Res 186:288-298; Tanaka A, Miyamoto K, Minamino N, Takeda M, Sato B, Matsuo H, Matsumoto K (1992) Proc Natl Acad Sci USA 89, 8928-32, Laboratory Note Book I/91

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