Promega Notes Magazine **Number 38, August 1992, p. 18.**

Comparison of attachment factors using the CellTiter 96^{TM} Assay

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Cell attachment assays can be easily performed and quantitated with the CellTiter 96 Non-Radioactive Cell Proliferation/Cytotoxicity Assay. The method involves fewer steps and less hands-on time than commonly used attachment assays, and data is collected at visible wavelength using a microplate reader.

Introduction

The measurement of cell attachment to coated surfaces is commonly used to study extracellular matrix (ECM) interactions and to determine which ECM coating is best for a specific cell type or particular application. Several methods are available for measuring viable attached cells. In general, each method begins with the coating of plastic tissue culture surfaces, such as 96-well plates, with different amounts of a given attachment factor. A suspension containing a known number of cells is then added to the wells and incubated at 37°C for about one hour to allow cell attachment. The unattached cells are washed away and the number of attached cells determined.

Common attachment assay methods

Probably the most commonly used attachment assay method is to fix the attached cells to the plastic surface with paraformaldehyde and then stain the cells with Toluidine blue. After several washing steps, the number of cells can be counted using a microscope. This is usually time consuming and tedious. For larger numbers of samples, stain can be eluted from the cells with a detergent solution and the absorbance read on a microplate reader.

Other common methods of determining the number of attached cells employ tracers such as a radioisotope (usually ^{51}Cr) or a substrate cleavable by cellular esterases to form fluorogenic compounds. Both of these methods require incubating the cells with label or substrate and then washing unincorporated label or substrate away from the cells just prior to their addition to the assay plate. These washing and centrifugation steps may cause damage to the cells.

Disadvantages of using ^{51}Cr or other radioactive tracers include the precautions necessary for safe handling, the paperwork, and the cost of isotope tracking and waste disposal. The fluorometric method also has disadvantages. Fresh fluorogenic substrate must be prepared just prior to cell labeling, and an expensive 96-well fluorometer is required to collect data.

CellTiter 96 cell attachment assay

With the CellTiter 96 Assay, the number of viable, attached cells can be measured with fewer steps and

thus less hands-on time than with other attachment assays (Figure 1). After unattached cells are removed, culture medium is added back to the attached cells, 15µl of Dye Solution are added to each well, and the plate is returned to the 37°C incubator for 4 hours. During this incubation, the tetrazolium dye is converted into a colored formazan precipitate that is directly proportional to the number of viable cells (1). After the incubation, 100µl of Solubilization Solution are added to each well to dissolve the formazan precipitate. Absorbance is recorded with a standard ELISA plate reader using a visible wavelength of 570nm. A more detailed protocol for this assay is available in Promega Technical Bulletin 122.

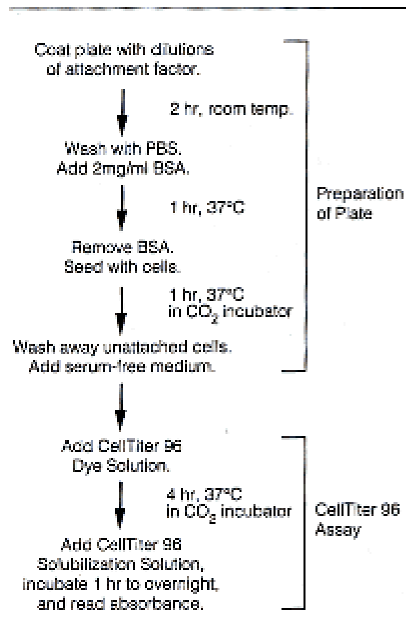

Figure 1. Cell attachment assay procedure using the CellTiter 96 Assay.

Results

We used the CellTiter 96 Assay to compare the attachment of BALB/3T3 fibroblasts to 96-well plates coated with one of three cell attachment factors supplied by Promega: Fibronectin, ProNectin™ F* and Vitronectin. In addition, cell attachment was evaluated on vitronectin supplied by another vendor. The concentration dependence of attachment was used to obtain a half-maximal concentration value for each factor (Figure 2).

*ProNectin F is a trademark of Protein Polymer Technologies, Inc. Patents pending.

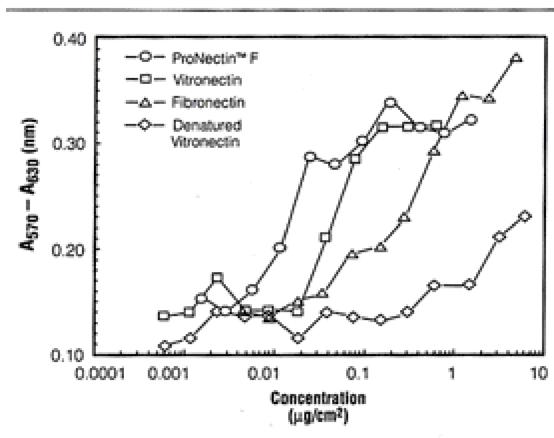

Figure 2. Comparison of cell attachment factors using the CellTiter 96 Assay. Plastic 96-well plates were coated with the indicated concentrations of attachment factors as described in Figure 1 and Technical Bulletin 122. Each well was seeded with 3.5 x 10^4 BALB/3T3 cells in serum-free medium. The plates were returned to the 37°C incubator for 1 hour, washed to remove unattached cells and then assayed using the CellTiter 96 Assay as described above. The absorbance was recorded with an ELISA plate reader at 570nm using 630nm as a reference wavelength.

Half maximal stimulation of attachment of BALB/3T3 cells in serum-free medium was obtained with the following concentrations of factors: ProNectin F = 0.02µg/cm^2, Vitronectin (Promega) = 0.04µg/cm^2, Fibronectin = 0.3µg/cm^2. Vitronectin supplied by the other vendor only supported attachment at the half maximal value when added at 6µg/cm^2, a concentration more than 100-fold higher than that needed with Promega Vitronectin.

Discussion

The greatest stimulation of cell attachment was seen with ProNectin F. This is not surprising, since ProNectin F is a polymer genetically engineered to promote cell attachment. It consists of 13 identical copies of the 10 amino acid cell attachment epitope from human fibronectin interspersed between structural peptide sequences. This 10 amino acid sequence (VTGRGDSPAS) contains the RGDS cell attachment epitope in a stable 3-dimensional conformation, making it highly resistant to thermal and chemical denaturation.

Vitronectin supplied by Promega exhibited much greater bioactivity than the Vitronectin obtained from another vendor (Figure 2). The most likely explanation for this difference is that Promega's Vitronectin is purified by a method which does not denature the molecule, whereas most commercial sources are denatured during purification (2).

References:

1. Mosmann, T. (1983) *J. Immunol. Meth.* **65**, 55.

2. Tomasini, B.R. and Mosher, D.F. (1991) In: *Progress in Hemostasis and Thrombosis Volume 10*. B.S. Collier, ed., W.B. Saunders Co.

Ordering Information:

CellTiter 96™ Non-Radioactive Cell Proliferation/Cytotoxicity Assay

Product	Size	Cat.#
CellTiter 96™ Non-Radioactive Cell Proliferation/CytotoxicityAssay	1,000 assays	G4000

Includes:

- 15ml Dye Solution
- 100ml Solubilization Solution
- 1 Protocol

Product	Size	Cat.#
CellTiter 96™ Non-Radioactive Cell Proliferation/Cytotoxicity Assay	5,000 assays	G4100

Includes:

- 75ml Dye Solution
- 500ml Solubilization Solution

Fibronectin, Human

Product	Size	Cat.#
Fibronectin, Human	1mg	G5291

ProNectin™ F Cell Attachment Polymer

Product	Size	Cat.#
ProNectin™ F Cell Attachment Polymer	1mg	G5411
	5mg	G5412

Vitronectin, Human

Product	Size	Cat.#
Vitronectin, Human	100µg	G5381

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