



- Cell Culture Applications

USE OF FLASHPLATE™ FOR CELL CULTURE

FlashPlate™ is a versatile tool which can be used for tissue culture applications. This bulletin contains general information, followed by specific examples for CHO cells, ³H-thymidine uptake, and a radioreceptor assay using human neutrophils. Appendix I contains a procedure for monitoring cell growth in tissue culture.

I. General Procedure

Materials:

- Basic FlashPlate (SMP-200)
- Plate Covers for Cell Culture
(available from NUNC - Cat. No. 264122, each cover is individually wrapped and sterile).
- 70% Ethanol or Sterile Media

Methods:

1. Cleaning

(Note: Basic FlashPlate is not sterile as provided.)

Basic FlashPlate can be cleaned prior to use by rinsing with sterile media or 70% ethanol. Plates rinsed in 70% ethanol have been shown to be clean enough to allow for culturing COS cells for at least 10 days(1). If using the ethanol rinse, air-dry Basic FlashPlate in a BioContainment Hood before adding any cells, as the ethanol is toxic to cells. Alternately, Basic FlashPlate may be rinsed with 70% ethanol and finally with sterile media immediately prior to use.

For some applications, such as culturing for less than 24-48 hours, no special treatment is needed(2). Additionally, antibiotics in the culture media prevent serious contamination in these short-term cultures.

2. Culture Conditions

A. Media

Standard media may be used with these plates; however, media containing colored pH indicators will have to be aspirated before counting due to color quenching.

B. Cell Growth

It is best to optimize the growth conditions for the cells in standard 96 well plates before using FlashPlate. Cells that do not normally attach to polystyrene culture dishes will probably not attach to FlashPlate. To establish whether cells have attached after culturing in FlashPlate, add trypsin or EDTA to remove the cells and count by standard methods. A quick visual inspection of the well after the media has been removed does show that the bottom of the well gets a matte finish when there is a good coverage of attached cells. When no cells are growing, the well bottom is very shiny. Alternately, a staining procedure can be used to visualize attached cells (see Appendix I).

C. Incubation

To facilitate culturing, use NUNC sterile plate covers (NUNC Cat. No. 264122) during incubations. Do not use these covers for counting. Immediately prior to counting switch to Top Seal-A Plate Covers (DuPont Cat. No. SMP201).

D. Non-adherent Cells

For the detection of radioactivity incorporated into or bound on the surface of non-adherent cells, centrifugation is necessary to pellet the cells prior to counting. It is not recommended to use ^3H in experiments with non-adherent cells.

3. Assay Conditions

A. Optimizing the Assay

When optimizing the assay, start with conditions which are known to work in other assays with the same cell line (i.e. filtration). It will probably not be necessary to increase the amount of radioactivity to detect binding.

B. Incubation

Use previously established incubation times as a starting point as well. If the incubation is at room temperature and you are using a colorless buffer or media, kinetic studies may be set up directly on the counter.

II. Protocol for Culturing CHO Cells Onto FlashPlate

A. Rinse FlashPlate with 70% Ethanol and air dry (optional if media contains antibacterial agents).

B. Prepare Media

DMEM: F-12 (1:1)
10% Fetal Calf Serum-heat inactivated
1% L-Glutamine
1% Penicillin Streptomycin
0.5 mg/ml G418 (dependent on your construct)

NOTE: Do not use CHO serum free media since it will promote cell growth in suspension.

C. Seed 3×10^3 to 4×10^4 cells per well in 100ul of media and incubate cells in $37^\circ\text{C}/10\% \text{CO}_2$ until confluent (approx. 1-2 days). Covers plates with Nunc covers (Cat. No. 264122).

When cells are confluent, the wells containing cells will have a rough surface and a dull reflection, as compared to an empty well.

There are different degrees of attachment (strong to weak) for various types of cells on FlashPlate. We found that CHO cells could withstand gentle aspiration (low negative force) and two rinses with media or PBS. In addition to CHO cells, we have had success growing CCL-64 (mink lung cell), A549 (human lung carcinoma) and various human breast cancer cell lines (i.e. T47D and BT549).

Cells can be stained using a toluidine Blue/glutaraldehyde procedure in order to visualize on FlashPlate. (See Appendix I)

Appendix I

Monitoring Cell Growth In Tissue Culture

Toluidine Blue/Glutaraldehyde Staining Procedure¹.

Materials

1. Glutaraldehyde 70% Aqueous Solution (Sigma, #G7776)
2. Toluidine Blue O (Sigma, #T0394)
3. Hanks Balanced Salt Solution (Sigma, #H8264)
4. Cells growing in FlashPlate

Methods (Applied to single wells or entire plate)

1. Remove the media by aspiration.
2. Dilute the glutaraldehyde to 5% in water and add to the wells.
3. Incubate for 10-15 minutes.
4. Rinse twice with water.
5. Add a 1% aqueous solution of Toluidine Blue O to the wells.
6. Incubate for 3-5 minutes.
7. Rinse once with water.
8. Air dry.

Note: For steps 2, 4, 5, and 7 the addition of solution and rinsing should be done gently so the cells will not become detached from the wells.

Results

The live cells will be visibly stained blue on the bottom of the wells, and the dead cells will not stay attached. The white FlashPlate eliminates the need to use a microscope to see the staining.

References

1. ATCC- Tissue Culture Staff (301-231-5585)