ECM Cell Culture Optimization Array (96-wells, Fluorometric)

Cat. No. ECM546

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures.

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Introduction

Extracellular matrix (ECM) proteins are produced intracellularly and are subsequently secreted into the surrounding cellular medium, actively regulating a diverse range of cell functions including cell adhesion, differentiation, proliferation, migration, invasion, and survival. ECM proteins are critical for *in vivo* and *in vitro* culture of a variety of cell types and are key building blocks of the normal 3D cellular environment. A primary utility of ECMs in *in vitro* culture is to promote cellular adhesion while maintaining cell viability and maximizing cell proliferation for downstream cell-based applications. In cases where optimal cell growth conditions are not well defined and critical adhesion protein requirements are unknown, identifying the ideal ECM protein(s) at functionally relevant concentration(s) can be a time-consuming and labor-intensive process. The **96-well ECM Cell Culture Optimization Array** is a multi-parametric assay that allows researchers to: (1) quickly identify ECM(s) that best promote cell-type specific adhesion, *and* (2) pinpoint the optimal ECM concentration required to promote maximum adhesion for a particular cell type.

The **96-well ECM Cell Culture Optimization Array** contains a 96-well microtiter plate where some of the most commonly used ECM proteins including collagen I, laminin, fibronectin and vitronectin are arrayed in duplicates at functionally relevant concentrations ranging from 0.125 µg/mL to 20 µg/mL. Also included in the kit are the necessary reagents to block, stain and analyze the cell adhesion capacity of cell types via a plate reader. The kit has been extensively tested and optimized on a number of different cell types including cancer cells, HEK293 cells, and Millipore's human and rodent neural stem cells. The kit is a rapid, sensitive, and reliable method to help researchers determine not only the best ECM for their specific cell type, but also the optimal ECM concentration to use for cell adhesion specific applications.

In addition to the featured products below, Millipore also provides numerous migration, invasion, and adhesion products including:

- 96-well ECM Cell Culture Optimization Array Kit (Colorimetric) (Catalog No. ECM541)
- 48-well ECM Cell Culture Optimization Array Kit (Colorimetric) (Catalog No. ECM542)
- Human Collagen Type I (Catalog No. CC050)
- Human Plasma Fibronectin Purified Protein (Catalog No. FC010)
- Purified Mouse Laminin (Catalog No. CC095)
- Purified Human Vitronectin (Catalog No. CC080)
- Accutase™ (SCR005)
- ReNcell Immortalized Cell Line (Catalog No. SCC008)
- CytoMatrix™ Screening Kit (Catalog No. ECM205)
- ECM Cell Adhesion Array Kit (Colorimetric) (Catalog No. ECM540)
- ECM Cell Adhesion Array Kit (Fluorimetric) (Catalog No. ECM545)
- Alpha/Beta Integrin-Mediated Cell Adhesion Array Combo Kit (Colorimetric) (Catalog No. ECM532)
- Alpha/Beta Integrin-Mediated Cell Adhesion Array Combo Kit (Fluorimetric) (Catalog No. ECM535)

- QCM™ 8µm 96-well Chemotaxis Cell Migration Assay (Catalog No. ECM510)
- QCM™ 5µm 96-well Chemotaxis Cell Migration Assay (Catalog No. ECM512)
- QCM™ 3µm 96-well Chemotaxis Cell Migration Assay (Catalog No. ECM515)
- QCM™ 96-well Cell Invasion Assay (Catalog No. ECM555)
- QCM™ 96-well Collagen-Based Cell Invasion Assay (Catalog No. ECM 556)
- 24-well Insert Cell Migration and Invasion Assay Systems

Test Principle

The Millipore ECM Cell Culture Optimization Array kit utilizes the fluorometric staining properties of Calcein-AM® for higher sensitivity (over colorimetric staining), as well as to allow for live cell imaging, and the quantitative detection of cell adhesion. Each kit contains a 96-well, microtiter plate (see layout on next page); each column (7 wells total) on the plate is pre-coated with decreasing concentrations of a specific ECM protein (20, 10, 5, 2.5, 1.25, 0.125 µg/mL) along with one BSA-coated well (negative control). Experimental cells are seeded onto the coated substrates, where adherent cells are captured. Subsequently, unbound cells are washed away, and the adherent cells are fixed and stained. After stain extraction, the relative cell attachment is determined using absorbance readings.

Additional information about the ECM components provided in the kit:

ECM Protein	Corresponding Millipore Cat. No.
Purified Human Collagen I	CC050
Purified Human Fibronectin	FC010
Purified Mouse Laminin	CC095
Purified Human Vitronectin	CC080

Application

Millipore's ECM Cell Culture Optimization Array kit is useful for assessing specific cell surface integrins, monitoring *in vitro* cell differentiation, or screening potential cell adhesion promoters/inhibitors.

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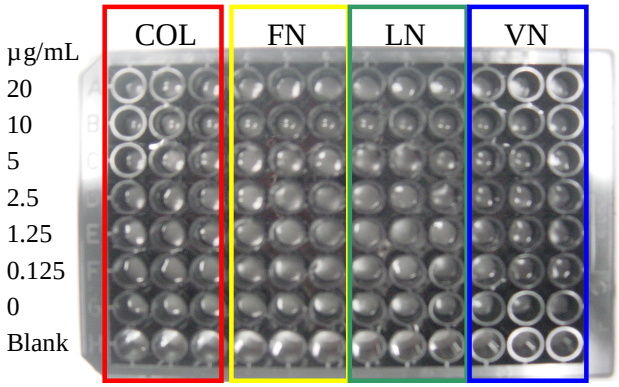
Kit Components

1. ECM Gradient Array Plate (Part No. CS201791): One 96-well plate. ECM proteins, collagen I (COL), fibronectin (FN), laminin (LN), and vitronectin (VN), are arrayed, respectively, in triplicates on the horizontal axis of the plate (see plate layout below). Vertically arrayed are the six different concentrations of the specified ECM proteins (20, 10, 5, 2.5, 1.25, and 0.125 µg/mL) along with the BSA-coated (negative control) and non-coated wells (blank control).
2. 30%BSA: (Part No. CS203352) One vial - 0.5mL
3. Calcein-AM: (Part No. CS202541) One vial – 50 µg. Ex/Em: 494/517nm.

ECM Array Plate Layout



Note: COL, FN, LN, and VN correspond to collagen I coated, fibronectin coated, laminin coated, and vitronectin coated wells, respectively.

Materials Not Supplied

- Multi-channel and/or repeating pipettes
- 15 mL or 50 mL conical tube.
- Harvesting buffer: 2.5 mM EDTA/PBS or Accutase (Catalog No. SCR005)
- Tissue culture growth medium appropriate for subject cells, such as DMEM containing 10% FBS
- Serum-free medium, such as DMEM, EMEM, or FBM (fibroblast basal media)
- Sterile 1X PBS or 1X HBSS to wash cells
- Deionized water
- Low speed centrifuge and falcon tubes for cell harvesting
- Cell culture incubator
- Sterile cell culture hood
- Hemacytometer and microscope for counting cells
- Trypan Blue or equivalent viability stain
- Microplate reader (517 nm detection) or spectrophotometer

Storage

The ECM Gradient Array Plate should be stored in the foil pouch at -70℃, and used within 3 months of the date of receipt. Special care should be taken to ensure that the desiccant remains in the pouch, and that the pouch is securely closed. (NOTE: In order to maintain the sensitivity of this kit, freeze/thaw cycles must be avoided).

Assay Instructions

1. Optimal assay timing and performance may vary for different cell lines, but generally can be obtained using subconfluent cell cultures. Subconfluent cultures can be achieved by splitting cells 1 to 2 days prior to performing the assay. Duplicate samples are suggested for greater accuracy.
2. Remove the ECM Cell Culture Optimization Array plate from the foil pouch and thaw at room temperature in a laminar hood. Carefully remove the top protection sealant while frozen. Upon thawing, there should be a thin layer of solution that evenly coats the array plate. Do not let the array plate dry out before you are ready to proceed to step 2.
3. Prepare Blocking Solution: Add 0.2 mL 30% BSA to 11.8 mL PBS in 15 mL conical tube. Mix thoroughly.
4. Prior to adding cells, remove the solution from the array plate by turning over the plate and gently tapping out the solution.
5. Immediately add 100 µL Block Solution to each well and incubate the array plate at room temperature for 1 hour.
6. In the meantime, prepare a single cell suspension, preferably using Accutase (Catalog No. SCR005) or a non-enzymatic dissociation buffer, such as PBS containing 2-5 mM EDTA for 10-20 minutes at room temperature. Optimum cell density may be determined by titration of the cells. A common starting range is 1- 2 x 10^6 cells/mL. When using PBS/EDTA adherent cells are often sticky. Gently pipette the cell suspension up and down through a 10 mL pipette; rinse several times with PBS/EDTA, use low speed spins, and do not over-spin, as cells will pack into clumps. Often it is best to harvest a greater number of cells than is needed. During the preparation let the large clumps settle to the bottom and extract the single cell suspension from the top of the tube.
7. Spin the final single cell suspension down. Aspirate the medium and gently resuspend the cells in serum-free media. Note: Serum-free media is defined as the basal medium that the cells are grown in (i.e. DMEM, EMEM, or FBM (fibroblast basal media)).
8. Repeat step 5 one to two more times to remove any residual serum.
9. After the last wash, resuspend the cell pellet to a range of 0.5- 2 x 10^6 cells/mL in serum-free medium.
10. After one hour of blocking (from step 3), discard the Block Solution from the wells by turning over the array plate and gently tapping out the solution. Tap the array plate on paper towels to dry the wells.

11. Add 100 µL of the cell suspension from step 7 to each well of the array plate, including BSA and Blank negative control wells. Incubate the plate for 30 to 120 min at 37℃ in a CO_2 incubator.

    Note: The amount of incubation time is cell-type dependent and should be optimally determined by the end-user.

10. After incubation, gently discard or aspirate the media from the wells (Note: do not allow the wells to dry). Gently wash each well 2-3 times with 200 µL per well of serum-free medium. When aspirating/removing the wash solution, try to leave about 25-50 µL per well so that the adherent cells remain hydrated at all times. After the third wash, inspect the array plate under a microscope to ensure that the wells have been sufficiently washed (i.e. there should be very little to no cells observed in the BSA-coated wells).

11. After washing, prepare Calcein-AM solution by adding 10 µL DMSO to the vial, vortex thoroughly, and quick spin-down the disperse droplets. Add 10 µL Calcein-AM solution to 10mL serum-free medium to make staining solution. Add 50µL staining solution to each well and incubate at 37 °C for 15 min.

12. Remove staining solution and wash each well with 200 µL serum-free medium 2-3 times. Discard the final wash and replace with 100 µL PBS for following analysis.

13. Determine the absorbance at 517 nm on a microplate reader or spectrophotometer. Alternatively, stained cells can be monitor via FITC channel.

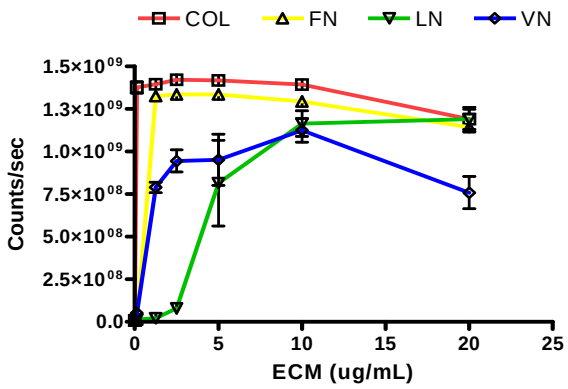
Calculation of Results

Results of the ECM Cell Culture Optimization Array Kit (Fluorometric) may be illustrated graphically using an ECM concentration gradient graph. A typical cell adhesion experiment compares the specific ECM-coated wells with their respective negative control well. Results from negative control wells are typically used as "Blanks" for interpretation of data. A small amount of background staining, or "noise," is expected from the Blank wells.

The following figures illustrate results obtained from different cell lines that have varying ECM requirements. One should use the data below for reference only. This data should **not** be used to interpret actual assay results.

HEK293 Cell Line:

A.



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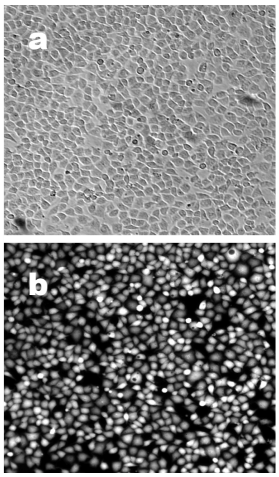
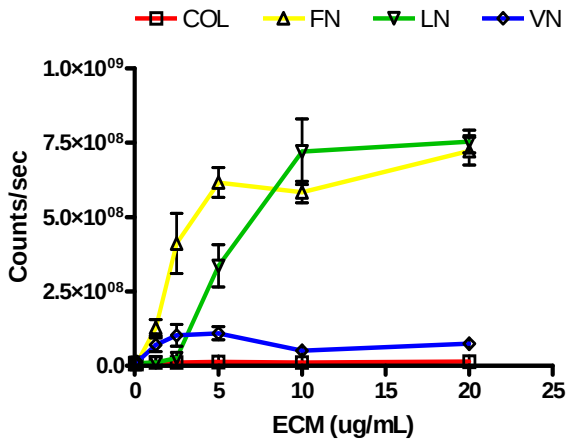


ReNcell VM Cell Line:

A.



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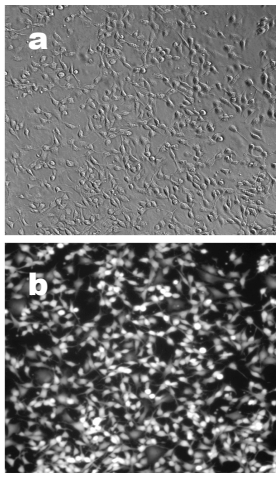

ECM Cell Culture Optimization Array Results. Cell attachment assay using HEK293 and ReNcell human ventral mesencephalon (ReNcell VM, Catalog No. SCC008) derived neural stem cells on ECM Cell Culture Array. 1 x 10^5 cells were added to each well and incubated for 2 hours at 37℃. BSA and Blank correspond to BSA coated neg ative control well and an uncoated blank control well. After incubation, wells were washed, stained, and measured as described under *Assay Instructions*. Representative graphs of the adhesive properties (A) and cell morphology by bright field microscopy (B,a) and fluorescence microscopy (B,b). COL: collagen (red), FN: fibronectin (yellow), LN: laminin (green) and VN: vitronectin (blue). Consistent with published literature, laminin and fibronectin were found in our assay to be the preferred substrates for human neural stem cells culture.

Warranty

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