User Manual
ECM540/Ver. 5.0/03Mar2023/rc

ECM Cell Adhesion Array Kit (Colorimetric)

ECM540

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures. Not for Human or Animal Consumption.

Product Overview

Cell adhesion plays a major role in cellular communication and regulation and is of fundamental importance in the development and maintenance of tissues. Scientists are continually examining the adhesion, migration, and invasion of many diverse cell types on various extracellular matrix (ECM) component proteins. The CHEMICON® ECM Cell Adhesion Array Kit (Colorimetric) provides an ECM Array microtiter plate with a colorimetric detection format, allowing for large-scale screening and quantitative comparison of multiple samples/cell lines. The kit is designed as a rapid (1-2 hours), cost effective, efficient method for characterization of cell adhesion.

In addition, Chemicon® continues to provide numerous migration, invasion, and adhesion products including:

- Alpha Integrin-Mediated Cell Adhesion Array Kit (Colorimetric) (Cat. No. ECM530)
- Beta Integrin-Mediated Cell Adhesion Array Kit (Colorimetric) (Cat. No. ECM531)
- Alpha/Beta Integrin-Mediated Cell Adhesion Array Combo Kit (Colorimetric) (Cat. No. ECM532)
- Alpha Integrin-Mediated Cell Adhesion Array Kit (Fluorimetric) (Cat. No. ECM533)
- Beta Integrin-Mediated Cell Adhesion Array Kit (Fluorimetric) (Cat. No. ECM534)
- Alpha/Beta Integrin-Mediated Cell Adhesion Array Combo Kit (Fluorimetric) (Cat. No. ECM535)
- ECM Cell Adhesion Array Kit (Fluorimetric) (Cat. No. ECM545)
- QCM™ 8µm 96-well Chemotaxis Cell Migration Assay (Cat. No. ECM510)
- QCM™ 5µm 96-well Chemotaxis Cell Migration Assay (Cat. No. ECM512)
- QCM™ 3µm 96-well Chemotaxis Cell Migration Assay (Cat. No. ECM515)
- QCM™ 96-well Cell Invasion Assay (Cat. No. ECM555)
- QCM™ 96-well Collagen-Based Cell Invasion Assay (Cat. No. ECM 556)
- 24-well Insert Cell Migration and Invasion Assay Systems
- CytoMatrix™ Cell Adhesion Strips (ECM protein coated)
- QuantiMatrix™ ECM Protein ELISA Kits

Test Principle

The CHEMICON® ECM Cell Adhesion Array kit utilizes a colorimetric detection format, allowing large-scale screening and quantitative comparison of multiple samples. Each kit contains a 96-well, microtiter plate consisting of 12 X 8-well removable strips. Each well within a strip (7-wells total) is pre-coated with a different ECM protein (see table below) 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 Chemicon® Cat. No.
Purified Human Collagen I	CC050
Purified Human Collagen II	CC052
Purified Human Collagen IV	CC076
Purified Human Fibronectin	FC010
Purified Human Laminin	AG56P
Purified Human Tenascin	CC065
Purified Human Vitronectin	CC080

Application

The CHEMICON® ECM Cell Adhesion Array kit is useful for assessing specific cell surface integrins, monitoring in vitro cell differentiation, or screening potential cell adhesion promoters/inhibitors.

Materials Provided

- ECM Array Plate: (Cat. No. 2003904) One 96-well plate with 12 strips. Each 8-well strip consists of 7 different human, ECM protein-coated wells (Collagen I, Collagen II, Collagen IV, Fibronectin, Laminin, Tenascin, Vitronectin) and one BSA-coated well (negative control). See the plate layout below.
- Cell Stain Solution: (Cat. No. 90144) One bottle - 20 mL.
- Extraction Buffer: (Cat. No. 90145) One bottle - 20 mL.
- Assay Buffer: (Cat. No. 2003646) One bottle - 100 mL.

ECM Array Plate Layout

Well Number

	1	2	3	4	5	6	7	8	9	10	11	12
A	Col I	Col I	Col I	Col I	Col I	Col I	Col I	Col I	Col I	Col I	Col I	Col I
B	Col II	Col II	Col II	Col II	Col II	Col II	Col II	Col II	Col II	Col II	Col II	Col II
C	Col IV	Col IV	Col IV	Col IV	Col IV	Col IV	Col IV	Col IV	Col IV	Col IV	Col IV	Col IV
D	FN	FN	FN	FN	FN	FN	FN	FN	FN	FN	FN	FN
E	LN	LN	LN	LN	LN	LN	LN	LN	LN	LN	LN	LN
F	TN	TN	TN	TN	TN	TN	TN	TN	TN	TN	TN	TN
G	VN	VN	VN	VN	VN	VN	VN	VN	VN	VN	VN	VN
H	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg

Note: Col I, Col II, Col IV, FN, LN, TN, VN, and Neg correspond to Collagen I, Collagen II, Collagen IV, Fibronectin, Laminin, Tenascin, Vitronectin, and BSA (Negative) coated wells, respectively.

Materials Required (Not supplied)

- Multi-channel and/or repeating pipettes.
- Harvesting buffer: EDTA or trypsin cell detachment buffer. Suggested formulations include 2 mM EDTA/PBS, 0.05% Trypsin in Hanks Balanced Salt Solution (HBSS) containing 25 mM HEPES, or other cell detachment formulations as optimized by individual investigators.

  Note: Trypsin cell detachment buffer may be required for difficult cell lines. Allow sufficient time for cell receptor recovery.
- Tissue culture growth medium appropriate for subject cells, such as DMEM containing 10% FBS.
- Quenching Medium: serum-free medium, such as DMEM, EMEM, or FBM (fibroblast basal media), containing 5% BSA.

  Note: Quenching Medium must contain divalent cations (Mg^{2+}, Ca^{2+}) sufficient for quenching EDTA in the harvesting buffer.
- Sterile PBS or HBSS to wash cells.
- Deionized water.
- Low speed centrifuge and tubes for cell harvesting.
- CO_2 incubator appropriate for subject cells.
- Sterile cell culture hood.
- Hemacytometer and microscope for counting cells.
- Trypan Blue or equivalent viability stain.
- Microplate reader (540-570 nm detection) or spectrophotometer.

Warnings and Precautions

- Cell stain contains minor amounts of crystal violet, a toxic substance, which may cause cancer and heritable genetic damage. Handle with caution. Toxic by inhalation and if swallowed. Irritating to eyes, respiratory system and skin.
- Extraction buffer contains alcohol. Avoid internal consumption.

Storage and Stability

The ECM Array Plate can be stored at 2 - 8 °C in the foil pouch up to its expiration date. Unused strips may be placed back in the pouch for storage. Ensure that the desiccant remains in the pouch, and that the pouch is securely closed. Keep the remaining kit components at 2 - 8 °C.

Protocol

1. Rehydrate the appropriate number of plate strips (see plate layout) with 200 µL of PBS per well for 10 minutes at room temperature.

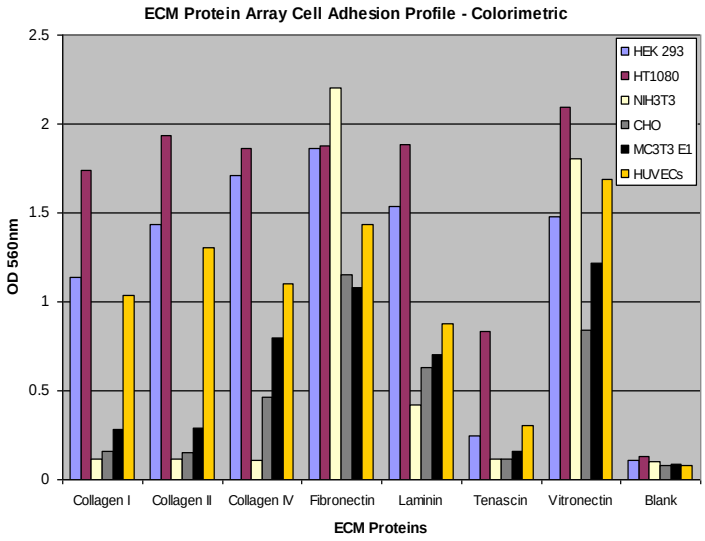
   Note: It is recommended that the entire strip be used for each assay.
2. Prior to adding cells, remove the PBS from the rehydrated strips by turning over the plate and gently tapping out the PBS.
3. Prepare a single cell suspension, preferably using a non-enzymatic dissociation buffer, such as PBS containing 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 1.0 to 2.0 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.
4. Spin the final single cell suspension down and gently resuspend in Assay Buffer or DMEM. One can also use a typical integrin binding buffer such as a solution of Hanks buffered salts solution, with 0.1% BSA, 25 mM HEPES, 1 mM Ca^{2+}/Mg^{2+}.
5. Add 100 µL of the cell suspension from step 4 to each well of the ECM Array Plate, including control well(s). It is recommended that you test each sample in duplicate or triplicate (requiring multiple strips). Incubate the plate for 1-2 hrs at 37 °C in a CO_2 incubator.
6. After incubation, gently discard or aspirate the media from the wells (Note: do not allow wells to dry). Gently wash each well 2-3 times with 200 µL per well of Assay Buffer. When aspirating/removing the wash solution try to leave about ~25-50 µL per well so the cells remain hydrated at all times.
7. After washing, add 100 µL of Cell Stain Solution to each well. Incubate for 5 minutes at room temperature. Remove the stain from the wells. Gently wash the plate 3-5 times with deionized water.
8. Discard the final wash and let the wells air dry for a few minutes.
9. Add 100 µL of Extraction Buffer to each well. Allow strips to incubate and gently rotate on an orbital shaker at room temperature until the cell-bound stain is completely solubilized, approximately 5-10 minutes.
10. Determine the absorbance at 540-570 nm on a microplate reader or spectrophotometer.

Data Analysis

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.

Results of the ECM Cell Adhesion Array Kit (Colorimetric) may be illustrated graphically using a "bar" chart. 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 chart illustrates typical results for the various cell lines. One should use the data below for reference only. These data should not be used to interpret actual assay results.



ECM Protein Cell Adhesion Colorimetric Array. HEK293, HT1080, NIH3T3, CHO, MC3T3-E1, and HUVEC cells were incubated for 2 hours at 37 °C. The negative control well contains BSA. 100 µL of the cell suspension at 150,000 cells per well was added. After incubation, wells were washed, stained, and measured as described under Assay Instructions.

Notice

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

The information in this document is subject to change without notice and should not be construed as a commitment by the manufacturing or selling entity, or an affiliate. We assume no responsibility for any errors that may appear in this document.

Technical Assistance

Visit the tech service page at SigmaAldrich.com/techservice.

Terms and Conditions of Sale

Warranty, use restrictions, and other conditions of sale may be found at SigmaAldrich.com/terms.

Contact Information

For the location of the office nearest you, go to SigmaAldrich.com/offices.

Document Template 20769660 Version 3.0

ECM540 Rev 03/23