



Integrin-Mediated Cell Adhesion Kit Combo Pack

96 Tests

Cat. No. ECM525

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

USA & Canada

Phone: +1(800) 437-7500 • Fax: +1 (951) 676-9209 • Europe +44 (0) 23 8026 2233
Australia +61 3 9839 2000 • Germany +49-6192-207300 • ISO Registered Worldwide
www.chemicon.com • custserv@chemicon.com • techserv@chemicon.com

Introduction

Cell adhesion plays a major role in cellular communication and regulation, and is of fundamental importance in the development and maintenance of tissues. Cell attachment to the extracellular matrix is mediated by a family of cell surface receptors, the integrins. Integrins are heterodimers containing an α and β subunit (1-2). Historically, antibodies have been used to determine the integrin profiles of cells by immunoprecipitation, immunofluorescence, immunoblotting, and flow cytometry. These methods are time-consuming, laborious or require sophisticated equipment. The CHEMICON Integrin-mediated Cell Adhesion Kit was designed as a cost effective, efficient method of cell surface integrin identification. The Kit is provided as 12 x 8-well removable strips in a plate frame for convenience and flexibility in designing assays. It offers a rapid and simple assay for the characterization of integrins on cells, being completed in 1-2 hours with little effort.

Test Principle

The CHEMICON Integrin-mediated Cell Adhesion Kit utilizes monoclonal antibodies generated against human $\alpha_v\beta_3$ integrin, $\alpha_v\beta_5$ integrin, or human β_1 , which are immobilized onto a goat anti-mouse antibody coated micro plate and used to capture cells expressing these integrins on their cell surface. A goat anti-mouse antibody coated microplate is provided as a negative assay control. Experimental cells are seeded onto the coated substrate and incubated. Subsequently, adherent cells are fixed and stained. Relative cell attachment is determined using absorbance readings.

Note: Monoclonal antibody to $\alpha_v\beta_3$ integrin, clone LM609, reacts with $\alpha_v\beta_3$ integrin from human, bovine, chick, monkey, porcine, canine, rabbit, and avian, but not samples from mouse or rat; Monoclonal antibody to $\alpha_v\beta_5$ integrin, clone P1F6, reacts with Human $\alpha_v\beta_5$ integrin; Monoclonal antibody to β_1 integrin, clone P4G11, reacts with Human β_1 integrin, and will interact with any alpha beta-1 heterodimer.

Application

The CHEMICON Integrin-mediated Cell Adhesion Kit can be used for assessing the presence or absence of specific integrins on the cell surface. This assay can substitute for FACS analysis (3) and is useful for screening human adherent cells for cell surface integrins or for monitoring *in vitro* cell differentiation or genetic modification of cells.

For Research Use Only. Not for use in diagnostic procedures.

Kit Materials

1. Integrin $\alpha_v\beta_3$ Capture Plate: (PN: 90123) One 96-well plate with 12 strips.
2. Integrin β_1 Capture Plate: (PN: 90122) One 96-well plate with 12 strips.
3. Integrin $\alpha_v\beta_5$ Capture Plate: (PN: 90124) One 96-well plate with 12 strips.
4. Control Plate: (PN: 90125) One 96-well plate with 12 strips containing only anti-mouse IgG.
5. Cell Stain Solution: (Part No. 90144) Two 20 mL bottles.
6. Extraction Buffer: (Part No. 90145) Two 20 mL bottles.

Materials Not Supplied

1. Multi-channel or repeating pipettes
2. Microplate reader (540-570 nm detection) or spectrophotometer
3. CO₂ incubator appropriate for subject cells
4. PBS to wash cells.
5. Distilled water

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

The experimental and control plates can be stored at 2-8°C in the foil pouch up to 6 months. 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.

Assay Instructions

1. Rehydrate the Integrin coated and uncoated control strips with 200 μL of PBS per well for 10 minutes at room temperature.
2. Gently remove the PBS from the rehydrated strips.
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 350,000 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 and during the preparation one lets the large clumps settle to the bottom and extracts the single cell suspensions near the top of the tube.
4. Spin the final single cell suspensions down one more time and gently resuspend in DMEM or PBS containing 1 mM Mn^{2+} , $\text{Ca}^{2+}/\text{Mg}^{2+}$. One can also use a typical integrin binding buffer such as a solution of Hanks buffered salts solution, with 0.1%BSA, 25mM HEPES, 1mM $\text{Ca}^{2+}/\text{Mg}^{2+}$.
5. Add 100 μL of cell suspension from step 4 to each well of the integrin capture plate and control plate. It is recommended that you test each sample in duplicate or triplicate. Incubate the plate for 1-2 hrs at 37°C in a CO_2 incubator.
6. After incubation, discard cells and remove residual media in the well by tapping the plate very **gently** upside down on a paper towel. **Gently** wash each well 2-3 times with PBS containing 1 mM $\text{Ca}^{2+}/\text{Mg}^{2+}$ (200 μL /well).
7. 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 PBS containing 1 mM $\text{Ca}^{2+}/\text{Mg}^{2+}$ (300 μL /well).
8. Discard the final wash and let the wells air dry.

9. Add 100 μ L 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 minutes.
10. Determine the absorbance at 540 - 570 nm on a microplate reader or spectrophotometer.

Calculation of Results

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.

Results of the integrin-mediated adhesion assay may be illustrated graphically by the use of a "bar" chart. A typical cell adhesion experiment will compare the integrin capture plate with the negative control plate and/or cells that do not express Integrin $\alpha_v\beta_3$. Results from negative control plate are typically used as "blanks" for interpretation of data. A small amount of background staining, or "noise" is obtained from the staining of the wells.

The following charts illustrate typical results for HT-1080, HUVEC and MRC 5 cells. One should use the data below for reference only. This data should not be used to interpret actual assay results.

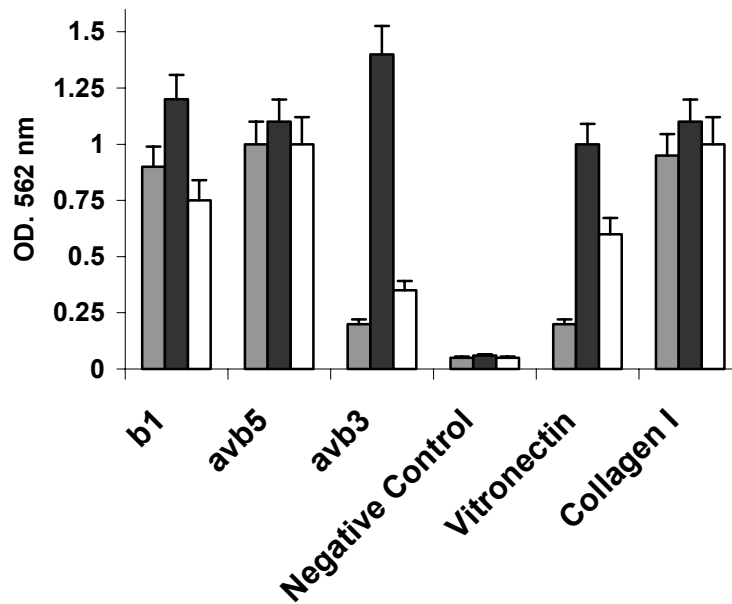


Fig. 1 Integrin-mediated Cell Adhesion Assay. HT-1080 fibrosarcoma cells (gray), MCR5 fibroblast cells (white) and HUVEC endothelial cells (black) were incubated for 1 hour at 37°C in wells coated with either ECM or integrin antibody. The negative control well contains only the secondary antibody for the integrin antibody. 100 μ L of cell suspension at about 350,000 cells/mL were added per well. After incubation, attached cells were stained and color measured as described under *Assay Instructions*.

References

1. Ruoslahti, E. (1991) *J. Clin. Invest.* **87**, 1-5.
2. Hynes, R.O. (1992) *Cell* **69**, 11-25.
3. Holmes, E. and Engvall, E. (1993) *Anal. Biochem.* **214**, 100-105.

Warranty

These products are warranted to perform as described in their labeling and in CHEMICON® literature when used in accordance with their instructions. THERE ARE NO WARRANTIES, WHICH EXTEND BEYOND THIS EXPRESSED WARRANTY AND CHEMICON® DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. CHEMICON®'s sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of CHEMICON®, to repair or replace the products. In no event shall CHEMICON® be liable for any proximate, incidental or consequential damages in connection with the products.

©2002: CHEMICON® International, Inc. - By CHEMICON® International, Inc. All rights reserved. No part of these works may be reproduced in any form without permissions in writing.

Cat No. ECM525

October 2002
Revision A: 41318