Collagen I Cell Adhesion Assay

Cat. No. CB012
(48 assays)

Introduction

Cell adhesion is a complex process involved in embryogenesis, migration/invasion, tissue remodeling, and wound healing. To perform these processes, cells adhere to extracellular matrix components (via adhesion receptors), forming complexes with components of the cytoskeleton that ultimately affect cell motility, differentiation, proliferation, and survival. Collagen I, a major structural component of extracellular matrices, is an excellent substrate for the culture of many different cell types. The Collagen I Cell Adhesion Assay is designed for the rapid, quantitative and reliable measurement of cell adhesion to collagen I. The kit includes a 48-well plate pre-coated with collagen I, as well as BSA (bovine serum albumin) as negative controls (see plate format in Figure 1). Cells are cultured in the pre-coated wells for a desired period of time, then unbounded cells are washed away, and the adhered cells are fixed and stained, followed by an extraction step which leads to dye elution from stained cells into supernatant. Thus cell adhesion can be quantified using a colorimetric ELISA plate reader at 595 nm.

Kit Components

Collagen Adhesion Plate: One 48-well plate containing 40 Bovine Collagen I coated wells and 8 BSA-coated wells (see layout below)
4X Lysis Buffer: 10.0 mL
CyQuant® GR Dye: 50 µL

Materials Not Supplied
1. Cell culture medium
2. Serum free medium, such as DMEM containing 0.5% BSA, 2 mM $CaCl_2$ and 2 mM $MgCl_2$
3. Cell culture incubator (37 ºC, 5% CO_2 atmosphere)
4. 1X PBS containing 2 mM $CaCl_2$ and 2 mM $MgCl_2$
5. Light microscope
6. 96-well plate suitable for a fluorescence plate reader
7. Fluorescence plate reader

Procedures

A. Under sterile conditions, allow the pre-coated 48-well plate to warm to room temperature and rinse once with PBS.
B. Seed cells of interest into the 48-well plate and culture for a desired period of time (at least 30-90 minutes) at 37°C.
C. After the culture is done, remove culture medium and rinse cells with PBS for 3-5 times.
D. Add 200 µl/well of freshly diluted 0.1% glutaraldehyde in PBS, fix for 10 minutes at room temperature. Then discard fixing solution and rinse cells 3 times with PBS.
E. Add 200 µl/well of Staining Solution, incubate for 30 minutes at room temperature on an orbital shaker.
F. After the staining is done, wash plate with DI water for 3-5 times. Pull off remaining wash water, invert plate onto an absorbent diaper pad and let the wells air dry.
G. Add 200 µl/well of Extraction Solution, incubate for 3-5 minutes and read OD_{595nm} using an ELISA plate reader.

	1	2	3	4	5	6	7	8
A	coll I	coll I	coll I	coll I	coll I	coll I	coll I	coll I
B	coll I	coll I	coll I	coll I	coll I	coll I	coll I	coll I
C	coll I	coll I	coll I	coll I	coll I	coll I	coll I	coll I
D	coll I	coll I	coll I	coll I	coll I	coll I	coll I	coll I
E	coll I	coll I	coll I	coll I	coll I	coll I	coll I	coll I
F	BSA	BSA	BSA	BSA	BSA	BSA	BSA	BSA

Figure 1. Layout of the pre-coated plate.

Usage

This assay kit is used to evaluate the collagen I cell adhesion *in vitro*. It is for research use only. Not for use in animals, humans, or diagnostic procedures.