Collagen I Cell Adhesion Assay

Cat. No. 8008
48 assays

Introduction

Cell adhesion plays an important role in cellular communication and regulation, and is of fundamental importance in the development and maintenance of tissues. Collagen I, a major structural component of extracellular matrices, is an excellent substrate for the culture of many different cell types. The ScienCell™ 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

Cat. No.	# of vials	Reagent	Volume	Storage
8008a	1	Collagen I & BSA coated 48-well plate	N/A	4°C
8008b	1	Staining Solution	10 ml	4°C
8008c	1	Extraction Solution	10 ml	4°C

Quality Control

Serial diluted Human Pulmonary Fibroblasts (Cat. No. 3300, ScienCell™) are cultured in the pre-coated 48-well plate. A linear relationship can be observed between signal produced (OD_{590nm}) and the number of cells, as shown in Figure 2.

Product Use

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.

Procedures

1. Under sterile conditions, allow the pre-coated 48-well plate to warm to room temperature and rinse once with PBS.
2. 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.
3. After the culture is done, remove culture medium and rinse cells with PBS for 3-5 times.
4. 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.
5. Add 200 µl/well of Staining Solution, incubate for 30 minutes at room temperature on an orbital shaker.
6. 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.
7. 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.

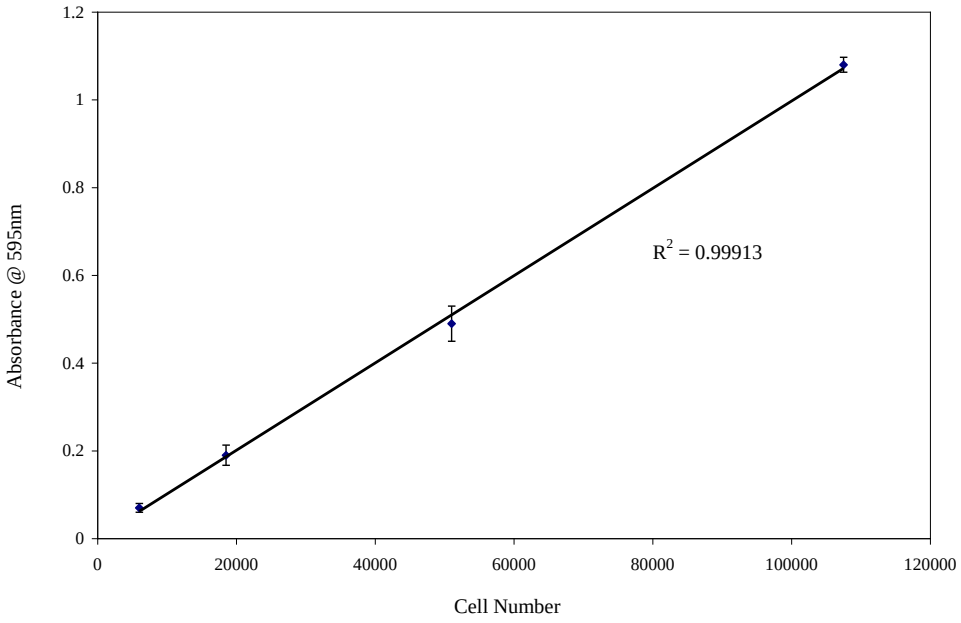

Figure 2. Correlation between cell number and absorbance at 595 nm shows linearity for human pulmonary fibroblasts.