Datasheet for ABIN5067547

CytoSelect™ 48-Well Cell Contraction Assay Kit

2 Images

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Overview

Quantity:	48 tests
Reactivity:	Mammalian
Application:	Cellular Assay (CA)

Product Details

Brand:	CytoSelect™
Sample Type:	Cell Samples
Characteristics:	CytoSelect™ 48-well Cell Contraction Assay Kit (Floating Matrix Model) provides a simple, in vitro system to assess cell contractivity and screen cell contraction mediators. The kit's proprietary Cell Contraction Plate eliminates the matrix releasing step of the conventional contraction assay, providing a faster, higher-throughput method to assess cell contraction. Each kit contains sufficient quantities to perform up to 48 assays in the provided 48-well plate.
Components:	1. 48-Well Cell Contraction Plate (Floating Model) : One 48-well plate (adhesion resistant matrix coated) 2. Collagen Solution : One 10 mL sterile bottle of bovine Type I Collagen at 3.0 mg/mL 3. Neutralization Solution : One 0.5 mL sterile tube 4. 5X DMEM Medium : One 5 mL sterile bottle 2 5. 5X PBS : One 5 mL sterile bottle 6. 100X Cell Contraction Inhibitor : One 1 mL sterile tube of 1M 2, 3-Butanedione Monoxime (BDM) in DMSO

Target Details

Background:	Wound healing is comprised of three processes: epithelialization, connective tissue deposition, and contraction. The contraction process is believed to be mediated by specialized fibroblasts

Target Details

called myofibroblasts. Three-dimensional collagen gels have been widely used to study fibroblast contraction, integrin signaling, cell apoptosis and cytoskeleton reorganization. Since three- dimensional matrix adhesions differ in structure, localization, and function from two-dimensional adhesions, and therefore, three-dimensional cell-matrix interactions may be more relevant biologically. The signaling mechanisms used by fibroblasts to regulate collagen matrix contraction depend on whether the cells are mechanically loaded or unloaded at the time that contraction is initiated as well as on the growth factor used to initiate contraction. For instance, stimulation of fibroblasts by lysophosphatidic acid (LPA) but not by platelet-derived growth factor (PDGF) causes robust force generation in restrained matrices, whereas LPA and PDGF stimulate floating matrix contraction equally well. There are several different culture models to study the ability of fibroblasts to reorganize and contract collagen matrices in vitro. In the attached model, a polymerized collagen matrix containing cells remains attached to a culture dish during contraction. Mechanical tension develops during contraction, and cellular stress fibers assemble. The two-step model combines an initial period of attached matrix contraction leading to mechanical loading, followed by release of the matrices, resulting in mechanical unloading and further contraction as mechanical stress dissipates. In the floating matrix contraction model, a freshly polymerized collagen matrix containing cells is released from the culture dish and allowed to float in culture medium, and contraction occurs in the absence of external mechanical load and without appearance of stress fibers in the cells.

Application Details

Application Notes:	Optimal working dilution should be determined by the investigator.
Comment:	• Proprietary contraction plate eliminates release step • Uses 3D collagen matrix to measure changes in collagen gel size • Includes optional cell contraction inhibitor
Assay Procedure:	1. Harvest cells and resuspend in desired medium at 2-5 x 106 cells/mL. 2. Prepare the cell contraction matrix by mixing 2 parts of cell suspension and 8 parts of cold Collagen Gel Working Solution. Note: Try to avoid introducing air bubbles to the mixture. Carefully mix by titurating the solution. Always include negative control wells that contain no cells in the matrix. 3. Add 250 µL of the cell contraction matrix to each well of the 48-well Cell Contraction Plate. 4. Transfer the plate to 37 °C and 5 % CO2 for 1 hour. 3 5. After collagen polymerization, carefully add 0.5 mL of culture medium (with/without contraction mediators) atop each collagen gel lattice. 6. Monitor wells for contraction over 2 days at 37 °C and 5 % CO2. Media should be changed daily by carefully removing 250 µL and replacing with 250 µL fresh media (with/without

Application Details

contraction mediators).

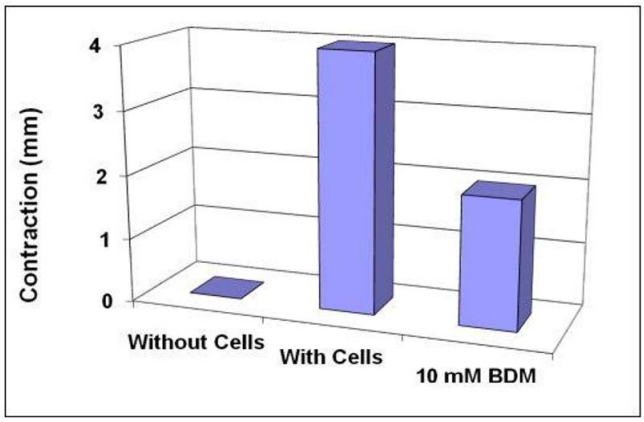
7. The collagen gel size change (contraction index) can be measured over time or as a set end point. Results can be quantified with a ruler or with image analysis software, such as NIH Image or Image Pro Plus.

Restrictions:	For Research Use only

Handling

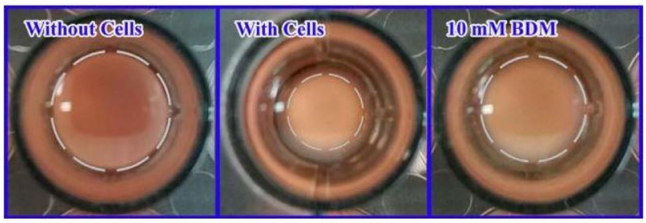
Storage:	4 °C
Storage Comment:	Store all components at 4°C. Preparation of Collagen Gel Working Solution This kit is designed for samples in a 48-well plate, and may be modified accordingly to suit other culture plate sizes. Keep all solutions at 4°C during assay preparation. Samples should be assayed at least in duplicate. 1. In a cold sterile tube, add the desired volume of Collagen Solution. Next, add corresponding 5X DMEM medium or 5X PBS to the tube, mix well. 2. Add Neutralization solution, IMMEDIATELY mix and keep the Collagen Gel Working Solution on ice. Note: Try to avoid introducing air bubbles to the mixture. Reagents 12 wells 24 wells 48 wells Collagen Solution 2.385 mL 4.77 mL 9.54 mL 5X Medium or PBS 615 µL 1.23 mL 2.46 mL Neutralization Solution 85 µL 170 µL 340 µL Total 3.085 mL 6.17 mL 12.34 mL

Images



Cellular Assay

Image 1. Contraction inhibition by BDM. 0.25 x 106 COS-7 cells in 250 µL collagen gel lattice were cultured for two days according to the Assay Protocol (top image). Dashed lines designate the gel edges. The change in matrix diameter size (in millimeters) was determined with a ruler



Cellular Assay

Image 2.