Datasheet for ABIN2344900

Collagen-based Cell Contraction Assay

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Overview

Quantity:	24 tests
Reactivity:	Mammalian
Application:	Cellular Assay (CA)

Product Details

Sample Type:	Cell Samples
Characteristics:	Collagen-based Contraction Assay Kit provides a simple system to assess cell contractivity in vitro and screen cell contraction mediators. Each kit provides sufficient quantities to perform up to 24 assays in a 24-well plate. The kit can also be used for culturing cells in a 3D collagen matrix. 2
Components:	1. Collagen Solution : One 10 mL bottle of sterile bovine Type I Collagen at 3.0 mg/mL 2. Neutralization Solution : One 0.5 mL tube 3. 5X DMEM Medium : One 5 mL bottle 4. 5X PBS : One 5 mL bottle 5. 100X Cell Contraction Inhibitor : One 1 mL tube of 1M 2, 3-Butanedione Monoxime (BDM) in DMSO
Material not included:	1. Cells such as Fibroblast 2. Cell culture medium 3. 37 °C Incubator, 5 % CO2 atmosphere 4. Sterile Spatula 5. Light microscope 6. Ruler 3

Target Details

Background:	Wound healing comprises of three processes: epithelialization, connective tissue deposition, and contraction. The contraction process is believed to be mediated by specialized fibroblasts called myofibroblasts. Three-dimensional collagen gels have been widely used in fibroblast contraction studies. There are several different culture models to study the ability of fibroblasts to reorganize and contract collagen matrices in vitro. In the floating 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. In the attached model, a polymerized collagen matrix containing cells remains attached to the 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. 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. 3D collagen matrix has also been used in the studies of 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.

Application Details

Application Notes:	Optimal working dilution should be determined by the investigator.
Comment:	• Uses a 3D collagen matrix to measure changes in the collagen gel size • Assess cell contractivity and screen for cell contraction mediators • 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 collagen lattice by mixing 2 parts of cell suspension and 8 parts of cold Collagen Gel Working Solution. 3. Add 0.5 mL of the cell-collagen mixture per well in a 24-well plate, incubate 1 hr at 37 °C. 4. After collagen polymerization, 1.0 mL of culture medium is added atop each collagen gel lattice. 5. Cultures are incubated for two days, during which stress develops. Before releasing the

Application Details

	stressed matrix, cells may be treated with contraction mediators, such as 10 mM BDM. To initiate contraction, gently release collagen gels from the sides of the culture dishes with a sterile spatula. 6. The collagen gel size change (contraction index) can be measured at various times with a ruler or quantified with image analysis software, such as NIH Image or Image Pro Plus. 4
Restrictions:	For Research Use only

Handling

Storage:	4 °C
Storage Comment:	Store all components at 4°C. 3 Preparation of Collagen Gel Working Solution This kit is designed for samples in a 24-well plate, and may be modified accordingly to suit other culture plate sizes. Keep all solutions ON ICE the entire time. 1. In a cold sterile tube, add the desired amount of Collagen Solution according to the table below. Next, add 5X DMEM medium or 5X PBS to the tube and mix well. 2. Add Neutralization solution, IMMEDIATELY mix and keep the Collagen Gel Working Solution on ice. Reagents 6 wells 12 wells 24 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

Publications

Product cited in:	Zhu, Jackson: "RACK1 regulates angiotensin II-induced contractions of SHR preglomerular vascular smooth muscle cells." in: **American journal of physiology. Renal physiology**, Vol. 312, Issue 4, pp. F565-F576, (2017) (PubMed). Jung, Song, Shin, Choi, Kim, Chung: "Local myogenic pulp-derived cell injection enhances craniofacial muscle regeneration in vivo." in: **Orthodontics & craniofacial research**, Vol. 20, Issue 1, pp. 35-43, (2017) (PubMed). Wu, Huang, Moore, Little, Walton, Fellner, Alexis, Peter Di, Redinbo, Tilley, Tarran: "Identification of BPIFA1/SPLUNC1 as an epithelium-derived smooth muscle relaxing factor." in: **Nature communications**, Vol. 8, pp. 14118, (2017) (PubMed). Lin, Zhen, Chien, Lee, Lin, Chen, Pai: "LIMCH1 regulates nonmuscle myosin-II activity and suppresses cell migration." in: **Molecular biology of the cell**, Vol. 28, Issue 8, pp. 1054-1065, (2017) (PubMed).

Halim, Hofstra, Signorile, Verdijk, van der Werf, Sribudiani, Brouwer, van IJcken, Dahl, Verheij, Baumann, Kerner, van Bever, Galjart, Wijnen, Tibboel, Burns, Muller, Brooks, Alves: "ACTG2 variants impair actin polymerization in sporadic Megacystis Microcolon Intestinal Hypoperistalsis Syndrome." in: **Human molecular genetics**, Vol. 25, Issue 3, pp. 571-83, (2016) (PubMed).

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