Product Manual

Cell Contraction Assay

Catalog Number

CBA-201 **24 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

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.

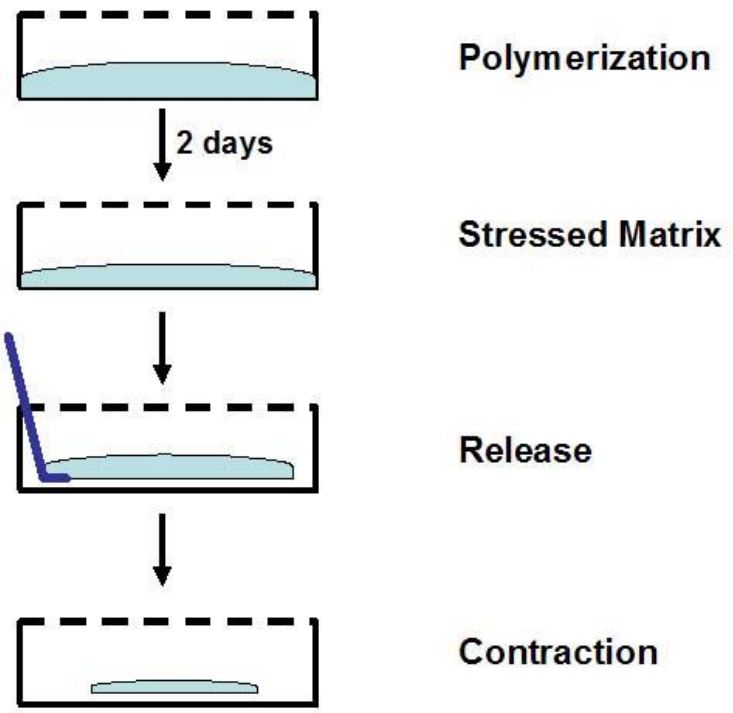
There are several different culture models to study the ability of fibroblasts to reorganize and contract collagen matrices in vitro. In the floating contraction model, a freshly polymerized collagen matrix containing cells is released from the culture dish and allowed to float in culture medium, and 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.

Cell Biolabs' 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.

Assay Principle



Kit Components (shipped on blue ice)

1. Collagen Solution (Part No. 20101): One 10 mL bottle of sterile bovine Type I Collagen at 3.0 mg/mL
2. Neutralization Solution (Part No. 20102): One 0.5 mL tube
3. 5X DMEM Medium (Part No. 20103): One 5 mL bottle
4. 5X PBS (Part No. 20104): One 5 mL bottle
5. 100X Cell Contraction Inhibitor (Part No. 20105): One 1 mL tube of 1M 2, 3-Butanedione Monoxime (BDM) in DMSO

Materials Not Supplied

1. Cells such as fibroblasts
2. Cell culture medium
3. 37ºC Incubator, 5% CO2 atmosphere
4. Sterile Spatula
5. Light microscope
6. Ruler

Storage
Store all components at 4ºC.

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.

Important Note: Be sure to pipet all volumes carefully with well-calibrated pipettes. Volumes of each reagent are critical for collagen polymerization.

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. *Note: Try to avoid introducing air bubbles to the mixture.*

	Number of wells in a 24-well plate		
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**

Assay Protocol (Two-Step Collagen Contraction Model)
1. Harvest cells and resuspend in desired medium at 2-5 x 10^6 cells/mL.
2. Prepare the collagen lattice 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 0.5 mL of the cell-collagen mixture per well in a 24-well plate, incubate 1 hr at 37ºC.
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 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.

Example of Results
The following figure demonstrates typical contraction results using the Cell Contraction Assay. One should use the data below for reference only. This data should not be used to interpret actual results.

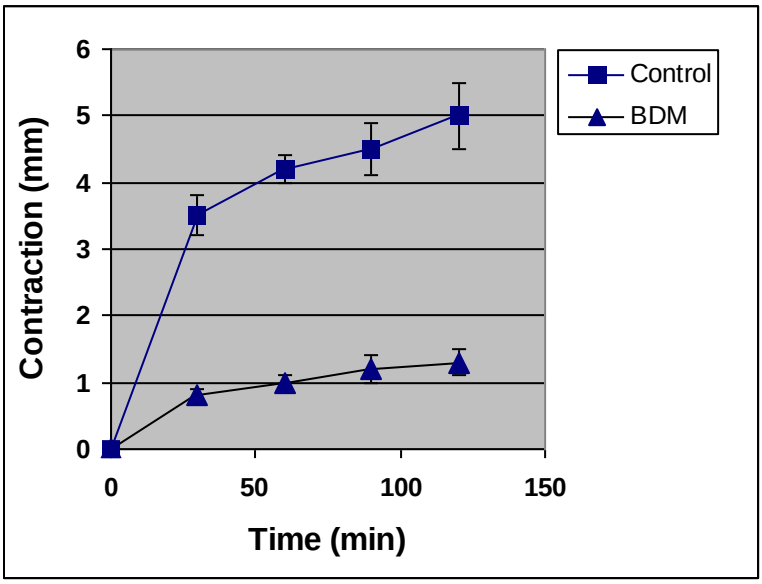


Figure 1. Contraction inhibition by BDM. 0.5 x 10^6 COS-7 cells in 0.5 mL collagen gel lattice were cultured for two days. Before initiation of contraction, cells were pretreated with 10 mM BDM for 1 hr. The change of gel size (diameter) in millimeters was measured with a ruler at various times after release.

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Recent Product Citations

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