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.

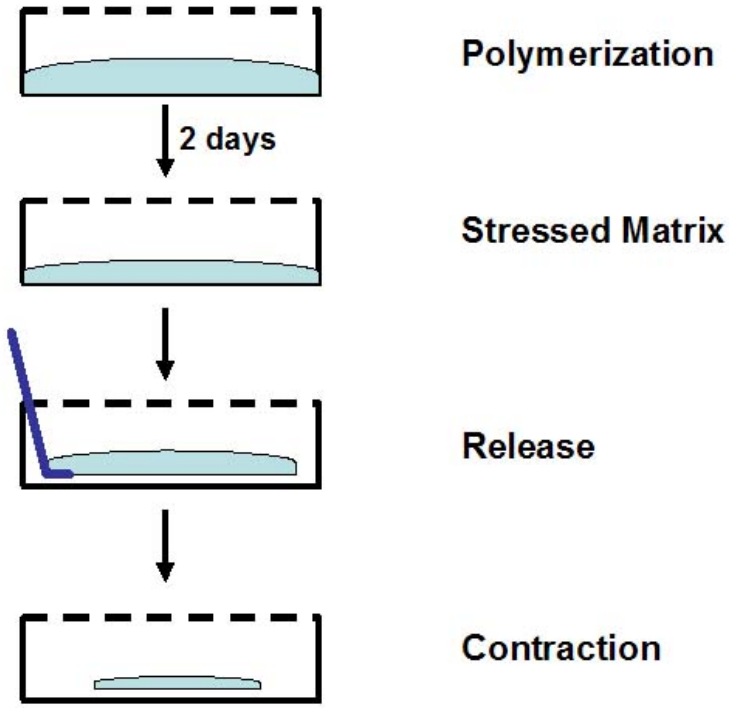
There are several different culture models to study the ability of fibroblasts to reorganize and contract collagen matrices in vitro. In the floating contraction model, a freshly polymerized collagen matrix containing cells is released from the culture dish and allowed to float in culture medium, and 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 24-well plate. The kit can be also used in culturing cells in 3D collagen matrix.

Assay Principle



Kit Components

1. Collagen Solution (Part No. 20101): One bottle – 10 mL of sterile bovine Type I Collagen at 3.0 mg/mL
2. Neutralization Solution (Part No. 20102): One tube – 1.0 mL
3. 5X DMEM Medium (Part No. 20103): One bottle – 5.0 mL
4. 5X PBS (Part No. 20104): One bottle – 5.0 mL
5. 100X Cell Contraction Inhibitor (Part No. 20105): One tube – 1 mL of 1M 2, 3-Butanedione Monoxime (BDM) in DMSO

Materials Not Supplied

1. Cells such as Fibroblast
2. Cell culture medium
3. 37^0 C Incubator, 5% CO2 atmosphere
4. Sterile Spatula
5. Light microscope
6. Ruler

Storage

Store all components at 4ºC until their expiration dates.

Preparation of Reagents

Collagen Solution: The chart below is suggested for samples in 24-well plate, and may be modified accordingly to suit other culture plate sizes. Keep all solutions ON ICE all the time.

1. In a cold sterile tube, add desired amount of collagen solution. Next, add 5X DMEM medium or 5X PBS to the tube, mix well.
2. Add Neutralization solution, IMMEDIATELY mix and keep the collagen gel 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**

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 collagen lattice by mixing 2 parts of cell suspension and 8 parts of cold collagen gel solution.
3. Add 0.5 mL of the cell-collagen mixture per well in a 24-well plate, incubate 1 hr at 37 ^{0}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 developed. Before release 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, 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.

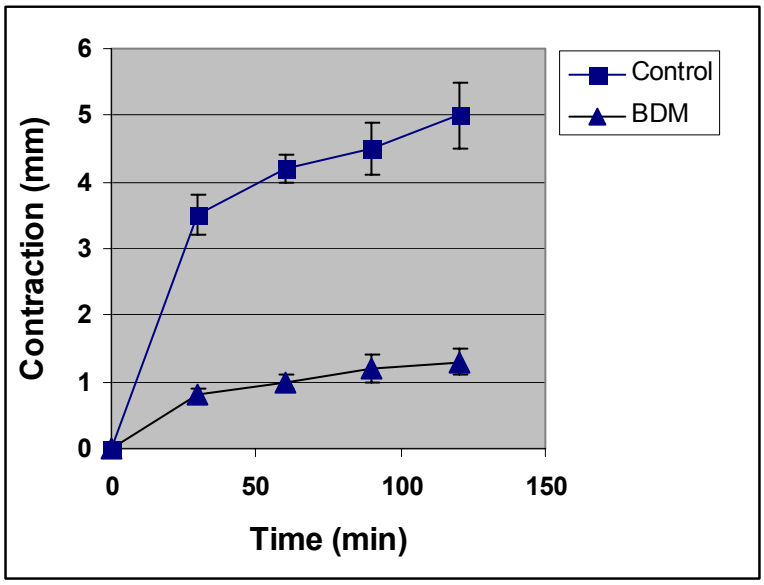


Figure 1. Contraction inhibition by BDM. 0.5 x 10^6 COS-7 cells in 0.5 mL collagen gel lattice were cultured for two days. Before initiation of contraction, cells were pretreated with 10 mM BDM for 1 hr. The change of gel size (diameter) in millimeters was measured with a ruler at various times after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

References

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4. Mochitate, K., Pawelek, P., and Grinnell, F. (1991) Exp. Cell Res. **193**, 198-207
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Warranty

Contact Information

Cell Biolabs, Inc.
7758 Arjons Drive
San Diego, CA 92126
Worldwide: +1 858-271-6500
USA Toll-Free: 1-888-CBL-0505
E-mail: tech@cellbiolabs.com
www.cellbiolabs.com