Product Manual

CytoSelect™ 48-Well Cell Contraction Assay Kit

Catalog Number

CBA-5021 **48 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

Introduction

Wound healing is comprised 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 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.

Cell Biolabs' 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.

Kit Components

Box 1 (shipped at room temperature)

1. 48-Well Cell Contraction Plate (Floating Model) (Part No. 50211B): One 48-well plate (adhesion resistant matrix coated)

Box 2 (shipped on blue ice packs)

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

Materials Not Supplied

1. Cells such as fibroblasts
2. Cell culture medium
3. Ruler

Storage

Store all components at 4ºC.

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 4ºC during assay preparation. Samples should be assayed at least in duplicate.

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 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.*

	Number of wells in a 48-well plate		
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**

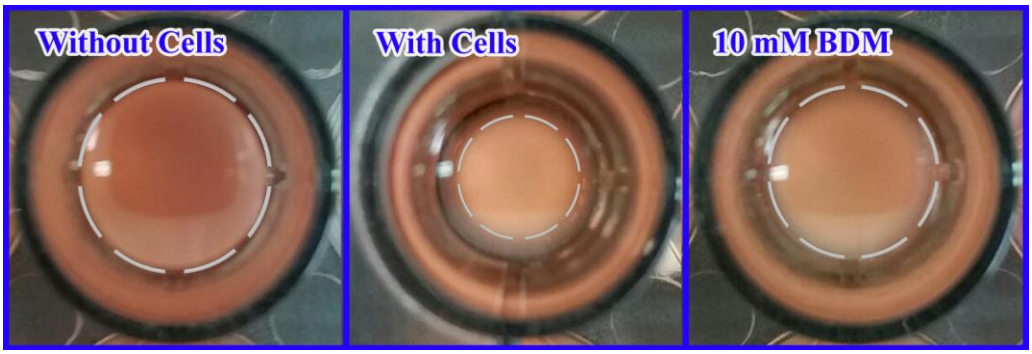
Assay Protocol (must be under sterile conditions)

1. Harvest cells and resuspend in desired medium at 2-5 x 10^6 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 37ºC and 5% CO_2 for 1 hour.
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 37ºC and 5% CO_2. Media should be changed daily by carefully removing 250 µL and replacing with 250 µL fresh media (with/without 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.

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.



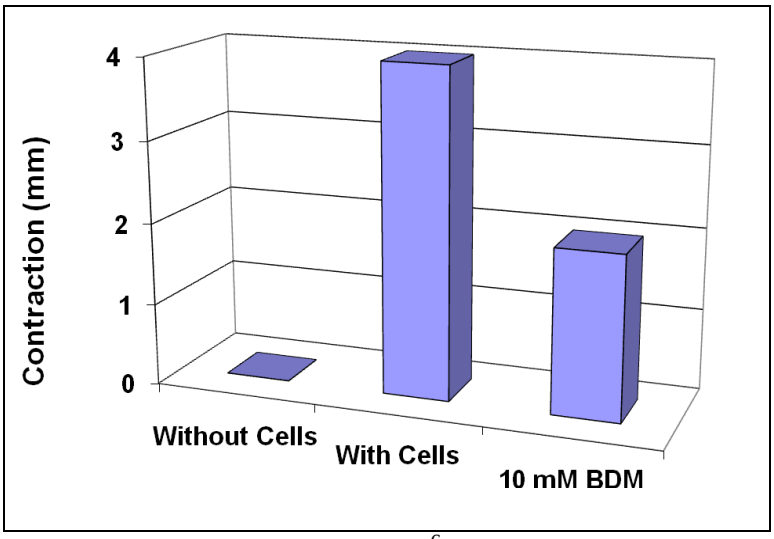


Figure 1. Contraction inhibition by BDM. 0.25 x 10^6 COS-7 cells in 250 µL collagen gel lattice was cultured for two days according to the Assay Protocol (top image). Dashed lines designate the gel edges. The change in matrix diameter size (in millimeters) was determined with a ruler.

References

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Recent Product Citations

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