ab325585 – *Collagen-based Cell Contraction Assay*

A simple system to assess cell contractivity *in vitro* and screen cell contraction mediators.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: www.abcam.com/ab325585

Storage and Stability: Store kit at +4°C immediately upon receipt. Refer to list of materials supplied for storage conditions of individual components.

Materials Supplied

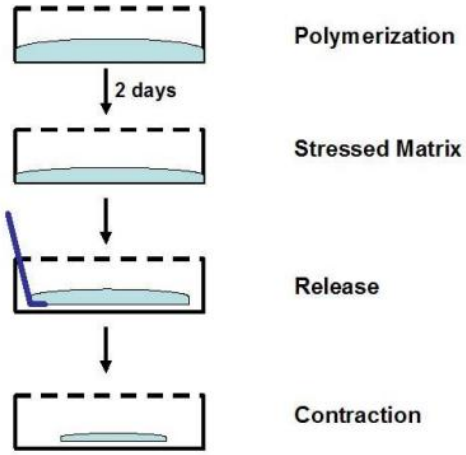
Item	Quantity 24 Tests	Storage Condition
Collagen Solution	10 mL	+4°C
Neutralization Solution	0.5 mL	+4°C
5X DMEM Medium	5 mL	+4°C
5X PBS	5 mL	+4°C
100X Cell Contraction Inhibitor	1 mL	+4°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Cells such as fibroblasts
- Cell culture medium
- 37°C Incubator, 5% CO_2 atmosphere
- Sterile Spatula
- Light microscope
- Ruler

Assay Principal



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 titrating 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 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 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.

Technical Support

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For all technical or commercial enquiries please go to:
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