Product Manual

CytoSelect™ 24-Well Cell Co-Culture System

Catalog Number

CBA-160	24 assays
CBA-160-5	5 x 24 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

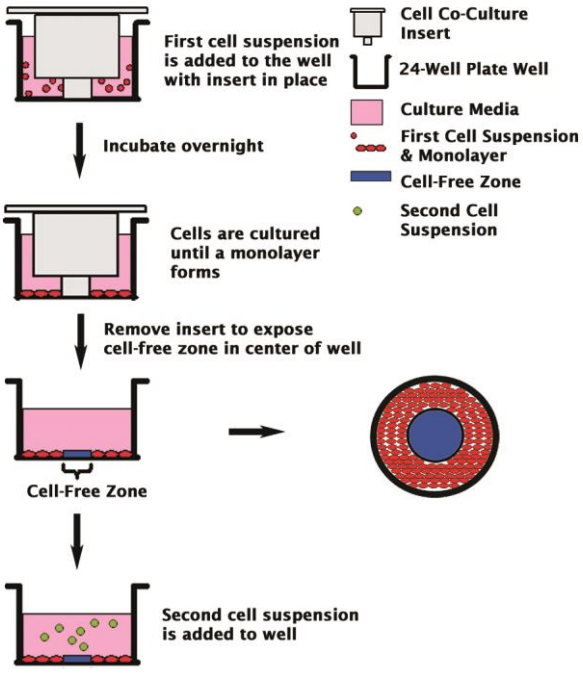
CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

The ability to co-culture multiple cell types has enabled novel ways to study cancer, developmental biology, and tissue engineering. Three-dimensional co-culture of endothelial and smooth muscle cells have enabled the structure of a blood vessel to be mimicked. Co-culture experiments of carcinoma and intratumoral stromal cells have played an important role in understanding breast cancer initiation, promotion, and progression. Additionally, feeder cells are commonly co-cultured with stem cells to maintain pluripotency.

A common co-culture method utilizes the Boyden Chamber to culture one cell type in the insert with a second cell type in the plate well. However, this approach does not allow for direct cell-cell interaction, recognized as critical in determining cell behavior and producing a more relevant *in vivo*-like environment. The Cell Biolabs CytoSelect™ 24-Well Cell Co-Culture System provides a unique platform to monitor direct contact between two cell types in a single well. First, cells are cultured until they form a monolayer around the insert, creating a defined 8 mm diameter cell-free zone. Once the insert is removed, a second cell type may be seeded into the exposed zone. The kit contains proprietary treated inserts and sufficient reagents for the evaluation of 24 samples. The inserts are compatible with most adherent cell types and experimental conditions.

The CytoSelect™ 24-well Cell Co-Culture System contains two 24-well plates each containing 12 proprietary treated plastic inserts. Calcein AM is also provided for viewing results with fluorescence microscopy.

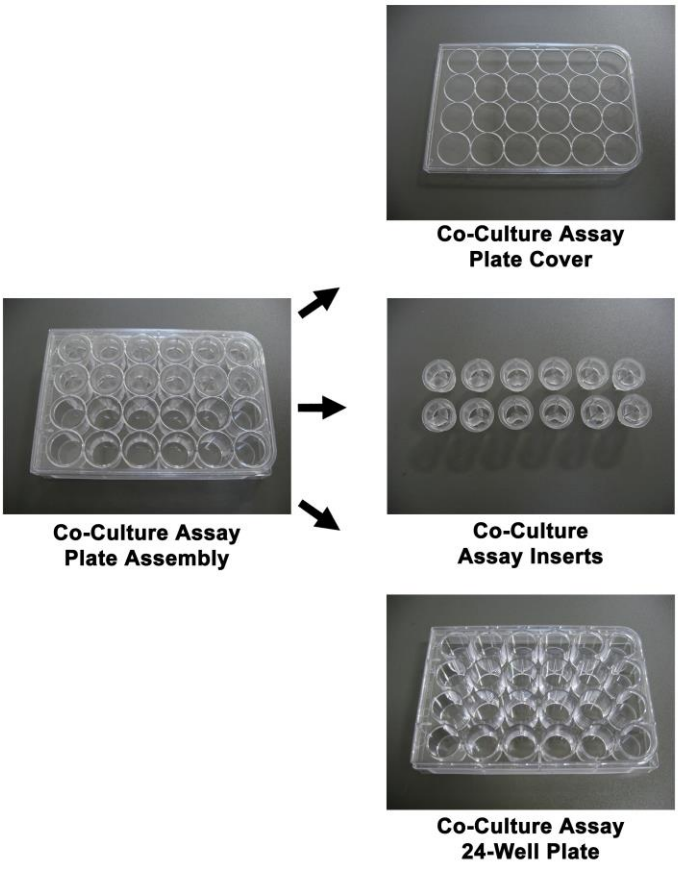


Related Products

1. CBA-100: CytoSelect™ 24-Well Cell Migration Assay (8 µm, Colorimetric)
2. CBA-106: CytoSelect™ 96-Well Cell Migration Assay (8 µm, Fluorometric)
3. CBA-120: CytoSelect™ 24-Well Wound Healing Assay (Microscopy)
4. CBA-125: Radius™ 24-Well Cell Migration Assay (Microscopy)
5. CBA-126: Radius™ 96-Well Cell Migration Assay (Microscopy)

Kit Components

1. 24-well Cell Co-Culture Plate Assembly (Part No. 116001): Two 24-well plates containing 12 inserts each (see Figure below)
2. Calcein AM (500X) (Part No. 108002): One vial – 50 µL in DMSO



Materials Not Supplied

1. Adherent cell lines and culture medium
2. Cell culture incubator (37ºC, 5% CO_2 atmosphere)
3. Inverted light microscope
4. Inverted fluorescence microscope
5. Forceps
6. PBS

Storage

Upon receipt, store the Cell Co-Culture Plates at room temperature and Calcein AM at -20ºC.

Preparation of Reagents

- 1X Calcein AM Solution: Just prior to use, prepare a 1X Calcein AM solution by diluting the provided stocks 1:500 in medium. Vortex thoroughly.

Protocol (Must be under sterile conditions)

Note: It is recommended that all samples be seeded in triplicate.

I. Initial Cell Seeding

1. Using sterile forceps, ensure that the inserts have firm contact with the bottom of the plate well.
2. Add 700 µL of culture medium to each well by carefully inserting the pipet tip through one of the top openings of the insert.
3. Harvest and resuspend cells in culture medium at 1 - 2 x 10^6 cells/ml.

   Note: Cell seeding density is highly cell line dependent, factoring in cell size, spreading and division. Ideally, the desired monolayer confluency should be 80-90%.
4. For optimal cell dispersion, add 75 µL of cell suspension to each of the three openings at the top of the insert, for a total of 225 µL per well. Take care to avoid bumping and moving the inserts.
5. Incubate cells in a cell culture incubator overnight or until a monolayer forms.
6. ***Carefully*** remove the insert from the well. Use sterile forceps to grab and lift the insert slowly from the plate well.
7. Slowly aspirate and discard the media from the wells. Wash wells with media to remove dead cells and debris. Finally, add media to wells to keep cells hydrated.

II. Secondary Cell Seeding

1. Harvest and resuspend cells in culture medium at 0.1 - 0.3 x 10^6 cells/ml.

   Note: Cell seeding density is highly cell line dependent, factoring in cell size, spreading and division. Ideally, the desired monolayer confluency should be 80-90%.
2. Carefully aspirate and discard the media from the wells. Do not allow wells to dry.

3. Slowly add 500 µL of the cell suspension to each well by carefully pipetting down the wall of the well.
4. Transfer the plate to a cell culture incubator for 4-24 hours to allow firm attachment/spreading. Take care to avoid shaking or bumping the plate.
5. Visualize wells under a light microscope. Repeat wash if wells still have debris or unattached cells.

(Optional) Calcein AM Fluorometric Labeling

1. Carefully aspirate and discard the media from the wells. Do not allow wells to dry.
2. Slowly add 500 µL of 1X Calcein AM solution to each well by carefully pipetting down the wall of the well.
3. Incubate the plate 30 minutes at 37°C.
4. Remove the Calcein AM and wash wells 2 times with medium.
5. After the last wash, add enough medium to cover the cells.
6. Monitor the cells microscopically for the presence of the green Calcein (Ex: 485 nm and Em: 515 nm) fluorescence.

Example of Results

The following figure demonstrates typical results with the CytoSelect™ 24-well Co-Culture System. This data should not be used to interpret actual results.

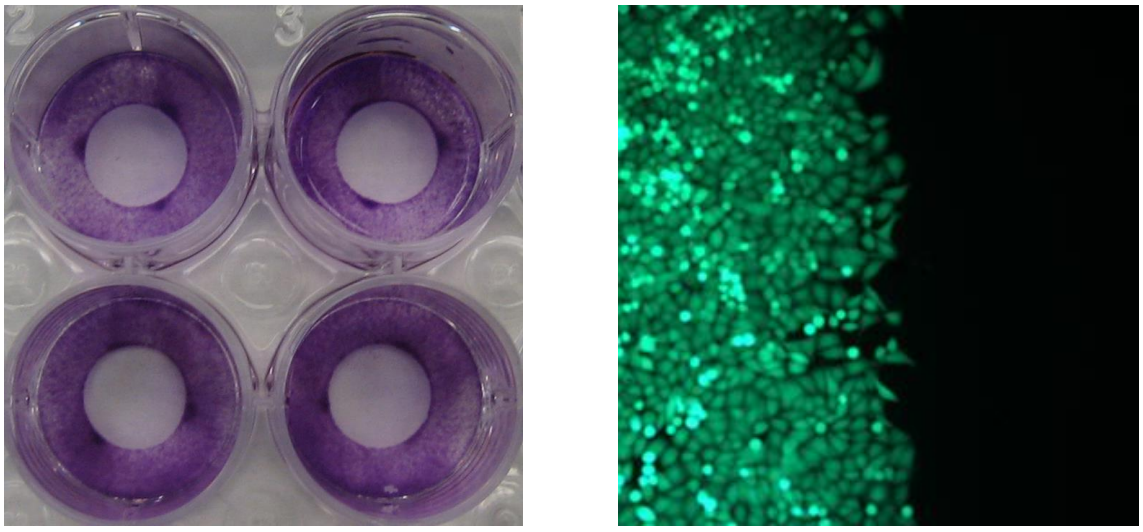


Figure 1: Defined Cell-free Zone. HeLa cells were seeded overnight according to the assay protocol. After insert removal, wells were stained with Crystal Violet (left) or Calcein AM (right) to demonstrate the cell-free zone.

References

1. Orlidge, A. and D'Amore, P.A. (1987) *J. Cell Biol.* **105**, 1455-1462.

2. Hesselman, M.C. et al. (2012) *PLoS One* **7**, e36982.
3. Hong, J.W. et al. (2013) *Lab Chip* **13**, 3033-3040.
4. Bacchus, W. et al. (2012) *Nat. Biotech.* **30**, 991-996.
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6. Campbell, J. et al. (2011) *PLoS One* **6**, e25661.

Recent Product Citations

1. Quick, Q.A. (2023). Efficacy of PP121 in primary and metastatic non-small cell lung cancers. Biomed Rep. 18(4):29. doi: 10.3892/br.2023.1611.
2. Jiang, L. et al. (2021). CRISPR activation of endogenous genes reprogramsfibroblasts into cardiovascular progenitorcells for myocardial infarction therapy. *Mol Ther*. doi: 10.1016/j.ymthe.2021.10.015.
3. Miyoshi, M. et al. (2019). LIM homeobox 2 promotes interaction between human iPS-derived hepatic progenitors and iPS-derived hepatic stellate-like cells. *Sci Rep*. **9**(1):2072. doi: 10.1038/s41598-018-37430-9.

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