Product Manual

CytoSelect™ 24-Well Cell Invasion Assay (Collagen I, Colorimetric Format)

Catalog Number

CBA-110-COL **12 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

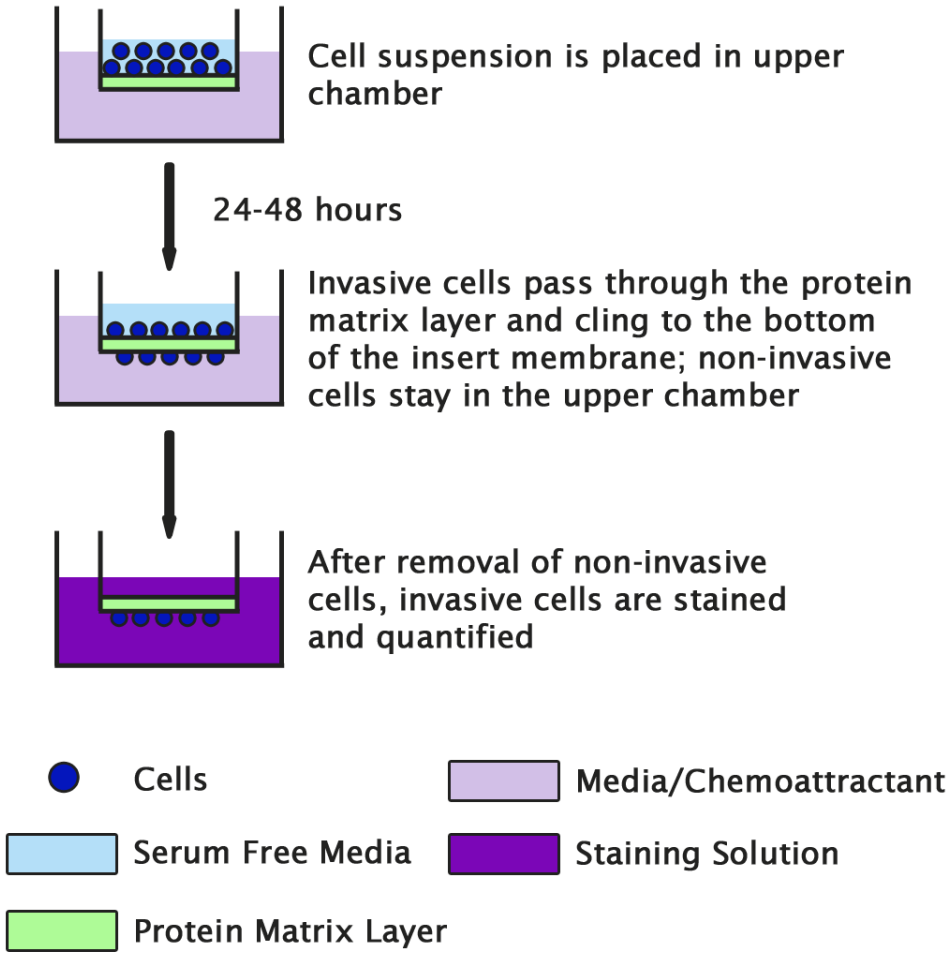
Introduction

The ability of malignant tumor cells to invade normal surrounding tissue contributes in large part to the significant morbidity and mortality of cancers. Invasiveness requires several distinct cellular functions including adhesion, motility, detachment, and extracellular matrix proteolysis. Metastatic cells produce many proteolytic enzymes (e.g. lysosomal hydrolysates, collagenases, plasminogen activators) while the expression of certain cell surface protease receptors is also increased.

Cell Biolabs CytoSelect™ Collagen Cell Invasion Assay Kit utilizes Bovine Type I Collagen-coated inserts to assay the invasive properties of tumor cells. It contains sufficient reagents for the evaluation of 12 samples.

Assay Principle

The CytoSelect™ Collagen Cell Invasion Assay Kit contains polycarbonate membrane inserts (8 µm pore size) in a 24-well plate. The upper surface of the insert membrane is coated with a uniform layer of dried Bovine Type I Collagen matrix. This collagen matrix layer serves as a barrier to discriminate invasive cells from non-invasive cells. Invasive cells are able to degrade the collagen matrix layer, and ultimately pass through the pores of the polycarbonate membrane. Finally, the cells are removed from the top of the membrane and the invaded cells are stained and quantified.



Related Products

1. CBA-100: CytoSelect™ 24-Well Cell Migration Assay (8µm, Colorimetric)
2. CBA-100-C: CytoSelect™ 24-Well Cell Migration and Invasion Assay (8µm, Colorimetric)
3. CBA-110: CytoSelect™ 24-Well Cell Invasion Assay (Basement Membrane, Colorimetric)
4. CBA-110-LN: CytoSelect™ 24-Well Cell Invasion Assay (Laminin I, Colorimetric)
5. CBA-111: CytoSelect™ 24-Well Cell Invasion Assay (Basement Membrane, Fluorometric)
6. CBA-111-COL: CytoSelect™ 24-Well Cell Invasion Assay (Collagen I, Fluorometric)
7. CBA-111-LN: CytoSelect™ 24-Well Cell Invasion Assay (Laminin I, Fluorometric)
8. CBA-112: CytoSelect™ 96-Well Cell Invasion Assay (Basement Membrane, Fluorometric)
9. CBA-112-COL: CytoSelect™ 96-Well Cell Invasion Assay (Collagen I, Fluorometric)
10. CBA-112-LN: CytoSelect™ 96-Well Cell Invasion Assay (Laminin, Fluorometric)
11. CBA-130: CytoSelect™ 96-Well Cell Transformation Assay (Soft Agar Colony Formation)

Kit Components

1. Collagen Invasion Chamber Plate (Part No. 111001-COL): One 24-well plate containing 12 collagen-coated cell culture inserts
2. Cell Stain Solution (Part No. 11002): One 10 mL bottle
3. Extraction Solution (Part No. 11003): One 10 mL bottle
4. Cotton Swabs (Part No. 11004): 40 each
5. Forceps: (Part No. 11005): One each

Materials Not Supplied

1. Invasive cell lines
2. Cell culture medium
3. Serum free medium, such as DMEM containing 0.5% BSA, 2 mM $CaCl_2$ and 2 mM $MgCl_2$
4. Cell culture incubator (37°C, 5% CO_2 atmosphere)
5. Light microscope
6. 96-well microtiter plate
7. Microtiter plate reader

Storage

Store all components at 4ºC.

Assay Protocol

1. Under sterile conditions, allow the collagen invasion chamber plate to warm up at room temperature for 10 minutes.
2. Rehydrate the collagen layer of the cell culture inserts by adding 300 µL of warm, serum-free media to the inner compartment. Incubate at room temperature for 30 minutes.
3. Prepare a cell suspension containing 0.5-1.0 x 10^6 cells/ml in serum free media. Agents that inhibit or stimulate cell invasion can be added directly to the cell suspension.

   Note: Overnight starvation may be performed prior to running the assay.
4. Carefully remove 250 µL of rehydration medium (step 2) from the inserts without disturbing the collagen layer (leaving 50 µL inside).
5. Add 250 µL of the cell suspension solution to the inside of each insert.
6. Add 500 µL of media containing 10% fetal bovine serum or desired chemoattractant(s) to the lower well of the invasion plate.
7. Incubate for 12-24 hours in a cell culture incubator.
8. Carefully aspirate the media from the inside of the insert. Wet the ends of 2-3 cotton-tipped swabs with water, flatten the ends of the swabs by pressing them against a clean hard surface, and gently swab the interior of the inserts to remove non-invasive cells. Take care not to puncture the polycarbonate membrane. Be sure to remove cells on the inside perimeter of the insert.
9. Transfer the insert to a clean well containing 400 µL of Cell Stain Solution and incubate for 10 minutes at room temperature.
10. Gently wash the stained inserts several times in a beaker of water. Allow the inserts to air dry.
11. (optional) Count invasive cells with a light microscope under high magnification objective, with at least three individual fields per insert.
12. Transfer each insert to an empty well, adding 200 µL of Extraction Solution per well, then incubating 10 minutes on an orbital shaker.
13. Transfer 100 µL from each sample to a 96-well microtiter plate and measure the OD 560nm in a plate reader.

Example of Results

The following figures demonstrate typical with the CytoSelect™ Collagen Cell Invasion Assay Kit. One should use the data below for reference only. This data should not be used to interpret actual results.

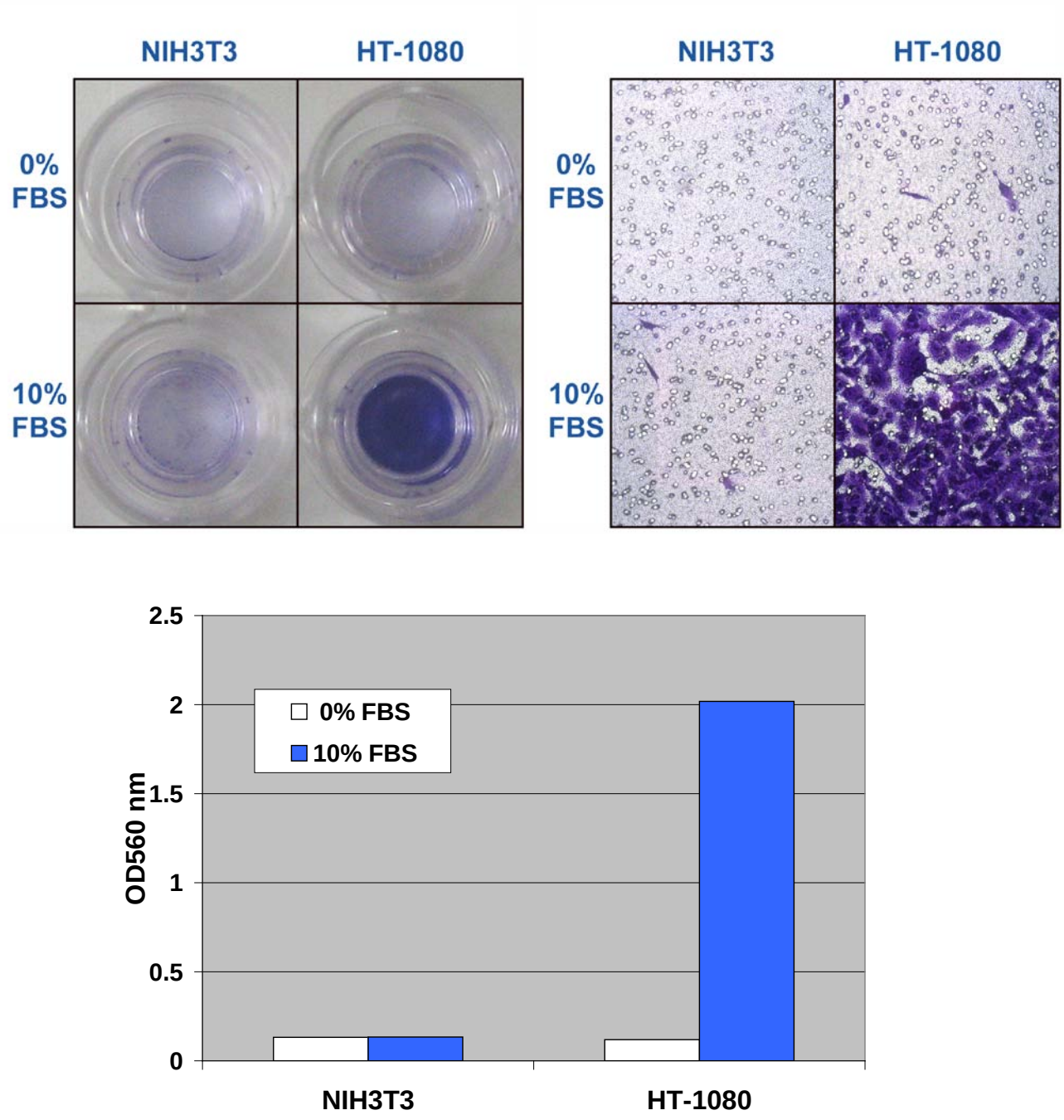


Figure 1. Human Fibrosarcoma HT-1080 Collagen Cell Invasion. HT-1080 and NIH3T3 (negative control) were seeded at 200,000 cells/well and allowed to invade toward FBS for 24 hrs. Invasive cells on the bottom of the invasion membrane were stained (top pictures) and quantified at OD 560nm after extraction (bottom panel figure).

References

1. Erkell, L. J., Schirrmacher, V. (1988) *Cancer Res* **48**, 6933-6937.
2. Montgomery, A. M. P., De Clerck, Y. A., Langley, K. E., Reisfeld, R. A., Mueller, B. M. (1993) *Cancer Res* **53**,693-700.
3. Monsky, W. L., Lin, C. Y., Aoyama, A., Kelly, T., Akiyama, S. K., Mueller, S. C., Chen, W. T. (1994) *Cancer Res* **54**,5702-5710.

Recent Product Citations

1. Djuzenova, C. S. et al. (2015). Actin cytoskeleton organization, cell surface modification and invasion rate of 5 glioblastoma cell lines differing in PTEN and p53 status. *Exp Cell Res.* **330**:346-357.
2. Mu, Y. et al. (2015). TGFβ-induced phosphorylation of Par6 promotes migration and invasion in prostate cancer cells. *Br J Cancer.* doi: 10.1038/bjc.2015.71.
3. Li, P. et al. (2015). A tight control of Rif1 by Oct4 and Smad3 is critical for mouse embryonic stem cell stability. *Cell Death Dis.* **6**:e1588.

Warranty

These products are warranted to perform as described in their labeling and in Cell Biolabs literature when used in accordance with their instructions. THERE ARE NO WARRANTIES THAT EXTEND BEYOND THIS EXPRESSED WARRANTY AND CELL BIOLABS DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. CELL BIOLABS' sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of CELL BIOLABS, to repair or replace the products. In no event shall CELL BIOLABS be liable for any proximate, incidental or consequential damages in connection with the products.

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