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APPLICATION NOTE

High-throughput cell migration assay with ThinCert® 96 Well HTS Insert

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ABOUT THIS APPLICATION NOTE

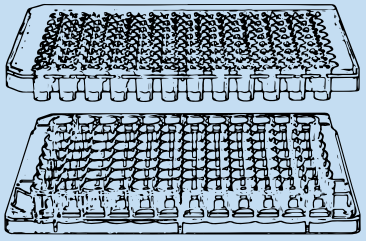
The ThinCert® 96 Well HTS Insert presents a novel high-throughput solution for conducting migration studies with unprecedented efficiency through the parallelisation and miniaturisation of experimental setups. The transparent porous membrane establishes consistent chemotactic gradients, providing researchers with the opportunity to delve into cell migration dynamics with improved precision and minimised experimental variability.

In this application note, we present a comprehensive step-by-step protocol detailing the utilisation of the HTS insert for migration studies emphasising its seamless integration into high-throughput workflows.

More information and recommended practices

KEY FACTS THINCERT® 96 WELL HTS INSERT

- **Optimal for migration and invasion assays**
- **96 well system for high-throughput applications**
- **Polycarbonate membrane with 3 µm and 8 µm pore size**
- **High transparency**
- **Precise fit of membrane plate and receiver plate for minimised wicking**



1. INTRODUCTION

Cell migration and invasion are fundamental processes that play a crucial role in numerous physiological and pathological events, such as morphogenesis [1,2], tissue repair[3], inflammation [4,5], and tumorigenesis [6,7]. The availability of in-vitro assays to study cell migration is of great importance for a better understanding of the underlying biological mechanisms and for the development of potential therapeutic interventions. Scientists leverage the close correlation between the in-vitro migratory potential of tumor cells and their in-vivo invasive properties [8]. This has elevated cell migration assays to a crucial tool in the study of novel anti-cancer medications. By capitalising on this relationship, researchers can effectively assess the efficacy and potential of various drug candidates.

So far a wide range of assays has been developed for studying cell migration, with the Boyden chamber assay being a commonly used in-vitro technique [9]. This assay utilises a two-compartment system in which cells are induced to migrate from an upper compartment through a porous membrane into a lower compartment, following the gradient of a chemoattractant. To ensure the assay's effectiveness, the size of the pores in the membrane must be selected carefully to prevent passive cell passage while allowing

active migration. Several modifications of the Boyden chamber assay have been developed, including variants that use extracellular matrix coatings for invasion assays, or a cell monolayer for transepithelial migration assays.

In this application note, we guide you through a high-throughput cell migration assay protocol with the new automation-compatible ThinCert® 96 well HTS insert. This system offers a highly efficient means of simultaneously testing a multitude of experimental conditions, effectively streamlining both time and resource utilisation, and significantly reducing the overall expenses associated with the assay. The optimised polycarbonate membrane creates stable chemotactic gradients while allowing for decent imaging due to its high clarity. These attributes synergistically make the ThinCert® 96 well HTS insert a perfect solution for large-scale motility studies.

2. MATERIALS AND METHODS

Item	Manufacturer	Item No.
Cells		
Human Connective Tissue Fibrosarcoma (HT-1080)	DSMZ	ACC 315
Michigan Cancer Foundation - 7 (MCF-7)	DSMZ	ACC 115
Cell culture media		
DMEM for HT-1080 cells	PAN Biotech	P04-04500
RPMI 1640 + non-essential AA + 1 mM Pyruvat for MCF-7	PAN Biotech	P04-18500
Cell culture chemicals	Sigma Aldrich	A7284-10ML
Trypsin/EDTA solution (0.05 %/0.02 %)	PAN Biotech	P10-023100
Fetal calf serum	PAN Biotech	P30-3306
Staining		
Calcein-AM	Sigma Aldrich	206700-1MG
Labware		
ThinCert® 96 well HTS insert (8 µm pore size)	Greiner Bio-One GmbH	655680
Microplate, 96 well, PS, F-bottom/chimney well, black, 10 pcs./bag	Greiner Bio-One GmbH	655076

3. ASSAY PRINCIPLE

The ThinCert® HTS insert is a tool designed to facilitate high-throughput experiments by providing a platform with 96 migration chambers. Each of these chambers is unique, as it consists of two distinct compartments, namely the upper compartment located within the membrane plate and the lower compartment found in the receiver plate. In order to conduct the assay, cells are seeded into the upper compartment and given the opportunity to adhere to the membrane.

Next, the chemotactic factor is placed into the lower compartment and passively diffuses through the membrane, inducing chemotaxis of the cells towards the chemoattractant source.

Following a calcein-AM fluorescence labelling of the cells, the migratory cells can be detached from the underside of the PC membrane and quantified using a standard fluorescence microplate reader.

Furthermore, a qualitative microscopic inspection can be conducted to detect the migrated fluorescence-labelled cells directly on the membrane.

3.1 SEEDING PREPARATION (GENERAL CELL CULTURE)

1. HT-1080 cells are cultivated according to standard cell culture protocols in serum-supplemented media
2. The day before the assay exchange the media with serum-free media (0.2 % BSA)
3. After cultivation overnight in serum-free media remove the cell media and rinse with PBS
4. Add trypsin solution to initiate cell detachment
5. Transfer the cell suspension to a tube and centrifuge at 350 x g for 5 min.
6. Resuspend the cell pellet in pre-warmed cell culture media
7. Dilute the cell suspension to a seeding density of 100,000 cells/ml

3.2 SEEDING CELLS IN THINCERT® 96 WELL HTS INSERT

1. Add 200 µl culture medium with or without chemoattractant (in this case various concentrations of FCS from 0.25 % to 10 %) to each well of the receiver plate
2. Add 50 µl of the above prepared cell suspension to each well of the membrane plate
3. Incubate the cell culture plate for 24 h in a cell culture incubator at 37 °C, 5 % CO_2)

3.3 CALCEIN STAINING

1. Remove the cell culture medium from each well of the receiver plate
2. Add 150 µl serum-free media with 8 µm Calcein-AM1 to the receiver wells
3. Incubate for 45 min in a cell culture incubator at 37 °C, 5 % CO_2

3.4 DETACHMENT AND MEASUREMENT IN THE READER

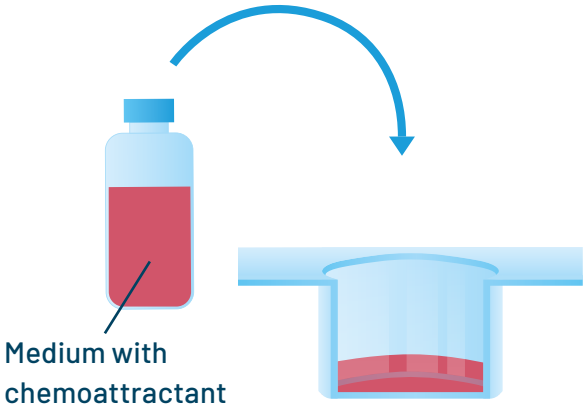
4. Remove the cell culture medium from each well of the membrane and the receiver plate
5. Rinse the wells of both plates with PBS
6. Add trypsin solution to receiver plate to initiate cell detachment of the migrated cells on the lower side of the membrane
7. Incubate the trypsin solution for 10 min. and shake gently.
8. Transfer 180 µl per well of the trypsin cell suspension to each well of the black 96 well microplate
9. Read fluorescence in a plate reader at an excitation wavelength of 485 nm and an emission wavelength of 520 nm

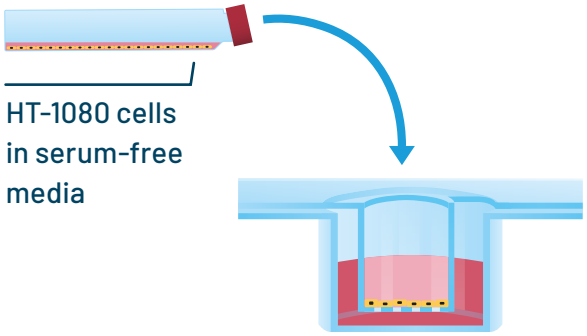
General remark: The media change can be performed through the access ports (Fig. 1), e.g., with a multi-channel pipette. Alternatively, the insert plate can be lifted and the media change of the receiver plate can be performed accordingly.

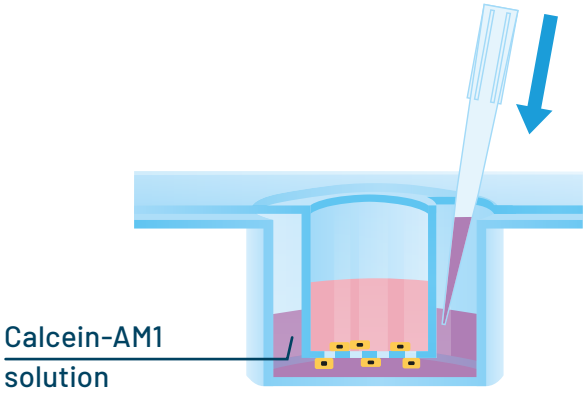
Useful hint:
Please handle the plate carefully to avoid excessive movement of the medium in the receiver plate.

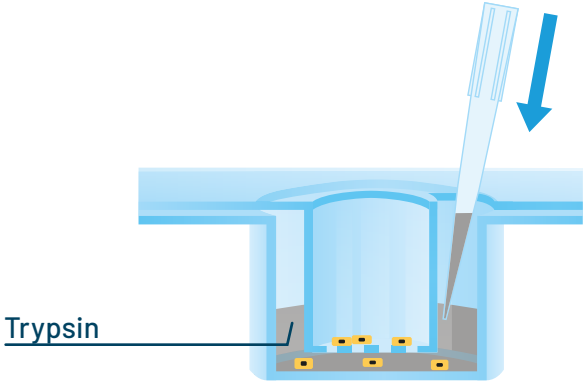
Figure 1: Access port is located next to the well and is accessible for (multi-channel) pipettes and automated liquid handling robots

4. WORKFLOW OF MIGRATION ASSAY









1 Assay preparation
Add medium with chemoattractant to receiver wells

2 Seeding cells
Add cell suspension to each well of the insert plate

Incubate the cell culture for 24 h

3 Calcein staining
Replace media within membrane and receiver plate with serum-free culture medium containing Calcein-AM1

Incubate the cell culture plate for 45 min.

4 Detachment
Aspire media from both compartments and add trypsin to receiver plate wells to initiate cell detachment

5. QUALITATIVE MICROSCOPIC DETECTION

Follow the instructions from 3.1 to 3.3 of the quantitative reader-based detection protocol. Instead of detaching the migrated cells from the basolateral side, the cells will be detected directly on the membrane. Therefore proceed with the following steps:

1. Aspirate the media from each well of the membrane plate
2. Use a pre-moistened cotton swab to remove the non-migrated cells on the upper (apical) side of the membrane by gently swirling the swab around the insert in a clockwise and counter-clockwise direction.
3. Detect the fluorescence signal of the migrated cells on membrane underside using a fluorescence microscope in the green channel

Furthermore, the ThinCert® 96 well HTS insert offers a high transparency due to its distinctive pore configuration. This characteristic brings substantial advantages by facilitating live cell observations for checking cell morphology, assessing cell confluence, and conducting contamination monitoring with enhanced accuracy and reliability. Each experiment was therefore accompanied by a continuous microscopic monitoring of the cells (Fig. 2).

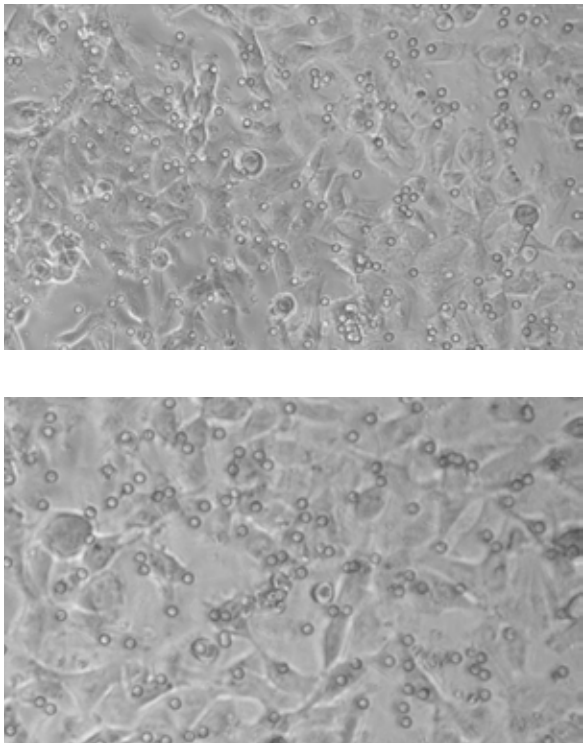


Figure 2: Live cell bright field microscopy of HT-1080 cells in ThinCert® 96 well HTS insert

6. RESULTS

In this study, we conducted a comprehensive investigation into the migratory behaviour of Human Connective Tissue Fibrosarcoma (HT-1080) cells in response to chemotactic factor gradients, specifically in the form of fetal calf serum (FCS), employing the protocol described earlier. The evaluation of cell migration was carried out through a multifaceted approach, combining quantitative analysis through fluorescence measurements with qualitative examination under the microscope.

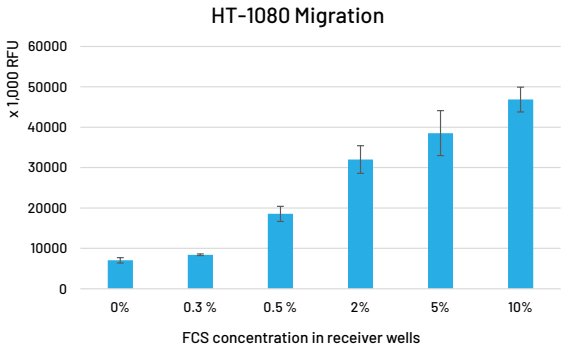


Figure 3: Migration of HT-1080 cells over 24 h in response to various FCS concentrations. Each data point represents the average of six wells that we run in parallel. Error bars indicate the standard deviation. (RFU: relative fluorescence units)

6.1 QUANTIFICATION OF MIGRATORY ACTIVITY

Upon seeding HT-1080 cells onto the membrane of the ThinCert® 96 well HTS insert, it was evident that their migration was influenced by the concentration of FCS gradients. The results indicate that the gradients with higher concentrations (i.e., 5 % or 10 % FCS in the lower chamber), exhibited more pronounced migration rates. Conversely, the gradients with weaker concentrations of 0.5 % FCS and 2 % FCS yielded comparatively lower migration rates (Fig. 3 and 5).

To further prove the system's functionality, low-migratory MCF-7 cells were employed as an additional control. As illustrated in figure 4, the average value in the 10 % FCS condition showed only a slight increase compared to the control with 0 % FCS.

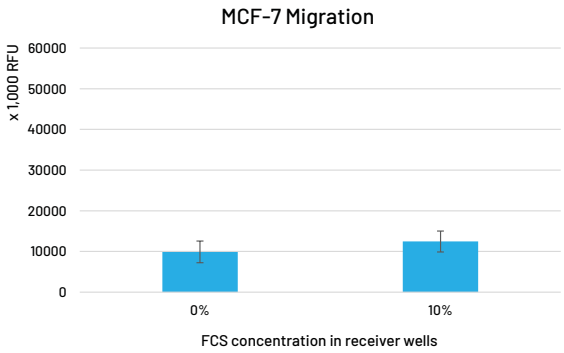


Figure 4: Migration of MCF-7 cells over 24 h to 0 % and 10 % FCS. Each data point represents the average of six well that we run in parallel. Error bars indicate the standard deviation. (RFU: relative fluorescence units)

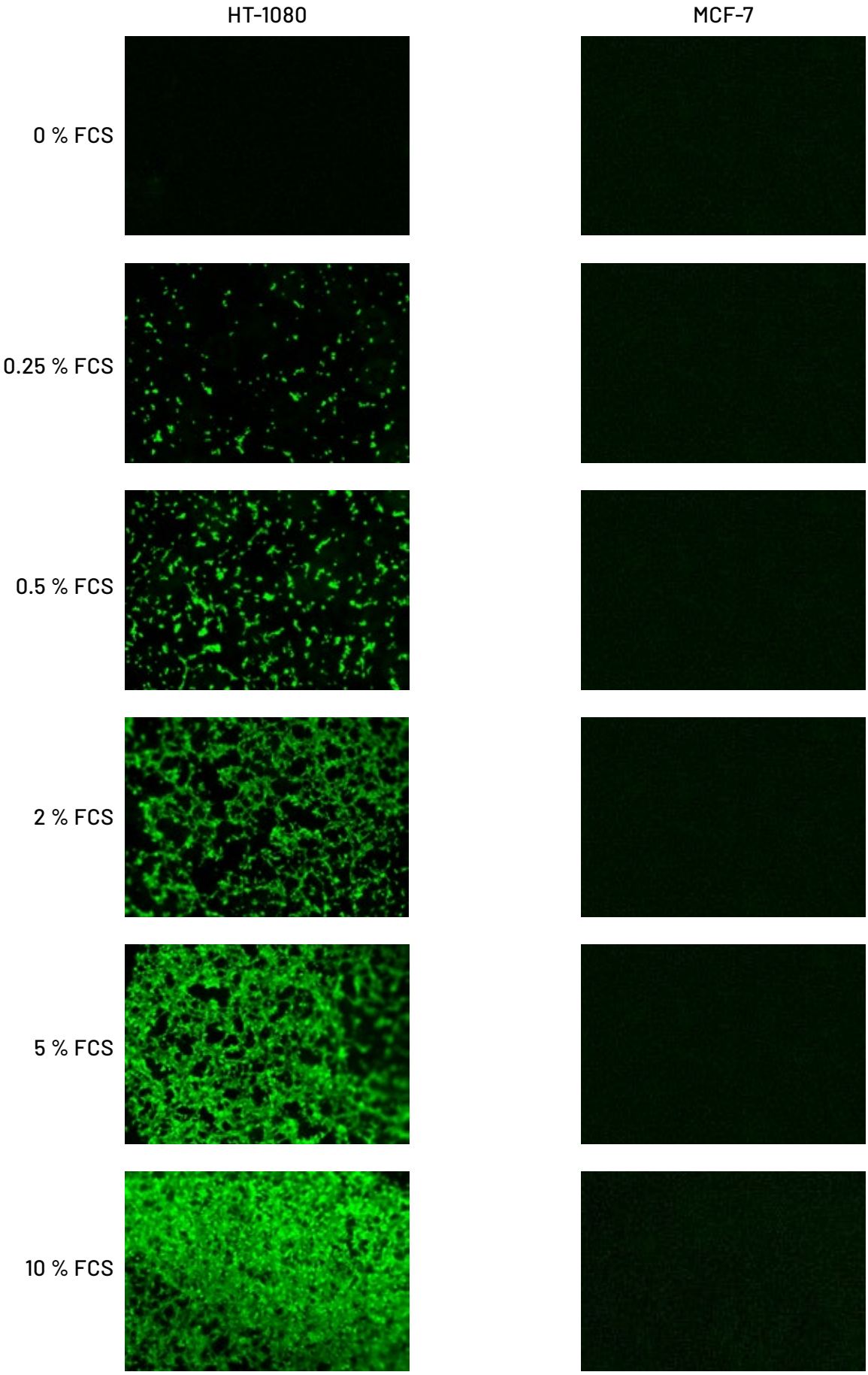


Figure 5: Microscopic inspection of the migratory behaviour of HT-1080 and MCF-7 cells after 24 h at different FCS concentrations. The green structures represent the fluorescence-stained cells at the underside of the membrane

6.2 QUALITATIVE MICROSCOPIC ANALYSIS

Furthermore, a qualitative visual analysis was performed using fluorescence microscopy. For this purpose, the non-migrated cells on the inner side of the membrane plate were gently removed with a wet cotton swab. The microscopic examination corroborates the findings from the quantitative analysis conducted in the microplate reader. The data clearly indicate a direct link between FCS concentration and enhanced migration of HT-1080 cells. As expected, the low-migratory MCF-7 cells do not show any significant migratory activity under the same conditions.

6.3 FINDING THE OPTIMAL SEEDING DENSITY

Optimising the seeding density for a specific cell line is crucial. If the concentration is too high, it becomes challenging to count the cells accurately, and it may also cause oversaturation of the pores in the membrane. On the other hand, adding too few cells can result in inconsistent counting and unreliable outcomes. Furthermore, as the seeding density rises, the likelihood of cells positioning themselves directly over membrane pores intensifies. At elevated concentrations, this phenomenon reaches a juncture where differentiating chemokinetic effects (random migration due to heightened motile activity) from chemotactic or haptotactic effects (migration prompted by the chemoattractant gradient) becomes increasingly intricate.

To investigate the migratory behaviour based on the seeding density, various cell numbers per well were examined. As shown in figure 6, across a wide range of cell populations (from 1.25×10^3 to 50×10^3 cells/migration chamber), the migratory HT-1080 cells exhibited a linear correlation with the total number of cells. It is recommended to use cell concentrations within this range when conducting a cell migration assay.

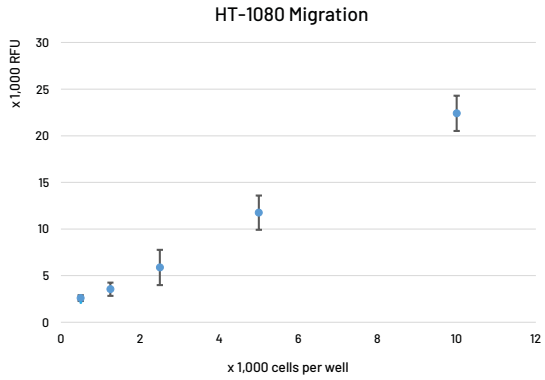


Figure 6: Migration of HT-1080 cells over 24 h in response to 10 % FCS. The relative fluorescence units (RFU) of migratory cells show a linear correlation with the total number of cells in the cell culture insert. Each data point represents the average of seven wells that we run in parallel. Error bars indicate the standard deviation. (RFU: relative fluorescence units)

7. CONCLUSION

In-vitro cell migration assays are indispensable tools in the quest for better biomarkers, therapeutic targets and agents to intervene in complex phenomena like cancer metastasis, angiogenesis and inflammatory diseases. They offer a controlled environment to measure cell migration and analyse the influence of different molecules, such as growth factors and chemokines, or drug candidates. The examples described in the application note illustrate the excellent suitability of ThinCert® 96 well HTS insert for cell migration assays.

The precise selection of pore size is crucial in motility experiments. It is essential that the pore size is proportionate to the dimensions of the studied cells, being both restrictive enough to prevent cells from moving passively and permissive enough to facilitate their active migration. Table 1 shows general membrane pore size recommendations.

Cell type	Example	Pore size recommendation (µm)
Epithelial cells	MCF7, MDA-MB-231	3
Endothelial cells	HUVEC, HMVEC	3
Polymorphonuclear neutrophils	-	3
Lymphocytes	T cells, B cells	3
Fibrosarcoma cells	NIH3T3, HT-1080	8
Leukocytes	Macrophages, Monocytes	8
Dendritic cells	BMDC	8

Table 1: Membrane pore size recommendations for common cell types

ORDERING INFORMATION

ThinCert® 96 Well HTS Insert (Membrane plates and receiver plates)

Growth area: 14 mm², Working volume (well of membrane plate): 15 - 160 µl, Working volume (well of receiver plate): 120 – 300 µl, Lid: yes, condensation rings

Item no.	Pore density	Ø Pores	Optical features of membrane	Surface treatment	Sterile	Qty. inner / outer
655630	2 x 10^6/cm²	3 µm	transparent	TC	+	1 / 5
655680	1 x 10^5/cm²	8 µm	transparent	TC	+	1 / 5

Receiver plates for ThinCert® 96 Well HTS Insert

Working volume (well of receiver plate): 120 – 300 µl, Lid: yes, condensation rings

Item no.	Growth area	Surface treatment	Sterile	Qty. inner / outer
655169	-	non-treated	+	8 / 32
655167	53 mm²	TC	+	8 / 32

INTERESTED IN OTHER APPLICATIONS FOR THINCERT® 96 WELL HTS INSERT?

Have a look at our application note
"**High-throughput in-vitro airway modelling with ThinCert® 96 well HTS insert**"

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REFERENCES

1. Friedl, P., Gilmour, D. Collective cell migration in morphogenesis, regeneration and cancer. Nat Rev Mol Cell Biol 10, 445–457 (2009). https://doi.org/10.1038/nrm2720
2. Aman A, Piotrowski T. Cell migration during morphogenesis. Dev Biol. 2010 May 1;341(1):20-33. doi: 10.1016/j.ydbio.2009.11.014. Epub 2009 Nov 13. PMID: 19914236.
3. Fu X, Liu G, Halim A, Ju Y, Luo Q, Song AG. Mesenchymal Stem Cell Migration and Tissue Repair. Cells. 2019 Jul 28;8(8):784. doi: 10.3390/cells8080784. PMID: 31357692; PMCID: PMC6721499.
4. Liu, J., Zhang, X., Cheng, Y. et al. Dendritic cell migration in inflammation and immunity. Cell Mol Immunol 18, 2461–2471 (2021). https://doi.org/10.1038/s41423-021-00726-4
5. Luster, A., Alon, R. & von Andrian, U. Immune cell migration in inflammation: present and future therapeutic targets. Nat Immunol 6, 1182–1190 (2005). https://doi.org/10.1038/ni1275
6. Justus CR, Leffler N, Ruiz-Echevarria M, Yang LV. In vitro cell migration and invasion assays. J Vis Exp. 2014 Jun 1;(88):51046. doi: 10.3791/51046. PMID: 24962652; PMCID: PMC4186330
7. Trepat X, Chen Z, Jacobson K. Cell migration. Compr Physiol. 2012 Oct;2(4):2369-92. doi: 10.1002/cphy.c110012. PMID: 23720251; PMCID: PMC4457291
8. Klemke R.L., Leng J., Molander R., Brooks P.C., Vuori K., Cheresh D.A. (1998) CAS/Crk coupling serves as a "molecular switch" for induction of cell migration. J Cell Biol. Feb 23;140(4):961-72
9. Boyden S. (1962) The chemotactic effect of mixtures of antibody and antigen on polymorphonuclear leucocytes. J Exp Med. Mar 1;115:453-66.

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