



GrowDex®

**GrowDex**

# **HIGH THROUGHPUT SCREENING GUIDE**

**How to reduce cost and time setting up reproducible  
HTS/HCS using 3D cell culture models?**

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## 1 Opportunities and challenges in 3D cell culture models for HTS and HCS

In drug development, high-throughput screening (HTS) is a commonly used method where the objective is to identify lead compounds with biological activity. The ability to do up to half a million test points in one day has opened new strategies in exploring the properties of these compounds. This technology enables more informed decisions regarding which compounds can be considered to move forward, as well as reducing the overall costs of drug development and decreasing the high failure rate in drug discovery.

In traditional two-dimensional (2D) cell culture methods cells grow on a flat tissue culture surface, whereas in a human body cells grow surrounded by other cells and the extracellular matrix in a three-dimensional (3D) environment. Therefore, 2D cell culture systems are a poor replication of human tissue outside the body. Models based on 3D cell culture better replicate the in vivo conditions by providing a more accurate representation of the in situ environment, allowing for complex cell orientation, dynamic cell-to-cell interactions, along with disease and injury modelling, making the cell culturing model more predictive. The 3D cell culture models can change how new drug molecules are tested in the pharmaceutical industry and potentially replace animal models in the drug development pipeline.

Many cell-based high-throughput screening (HTS) and high-content screening (HCS) assays are still done using traditional 2D cultures. The highly artificial HTS monolayer 2D cultures are thought to significantly impact the predictive value of compound screens which lead to high failure rates in drug discovery. Advanced, but simple to use 3D cell-based assays for HTS and HCS could work as a solution to provide more physiologically relevant cell culture models.

Although 3D culture models offer several advantages, there are still challenges which need to be overcome, such as scaling up 3D cell culture assays to higher throughput using automated dispensing or doing automated image analysis. The advantages and disadvantages of choosing different culture formats and technologies are shown in Table 1 and 2 respectively [1]. Schematic representation of 3D cell culture models with GrowDex® hydrogels, U-bottom Ultra Low Adhesion (ULA) plates and animal derived matrices are presented in Figures 1-3.

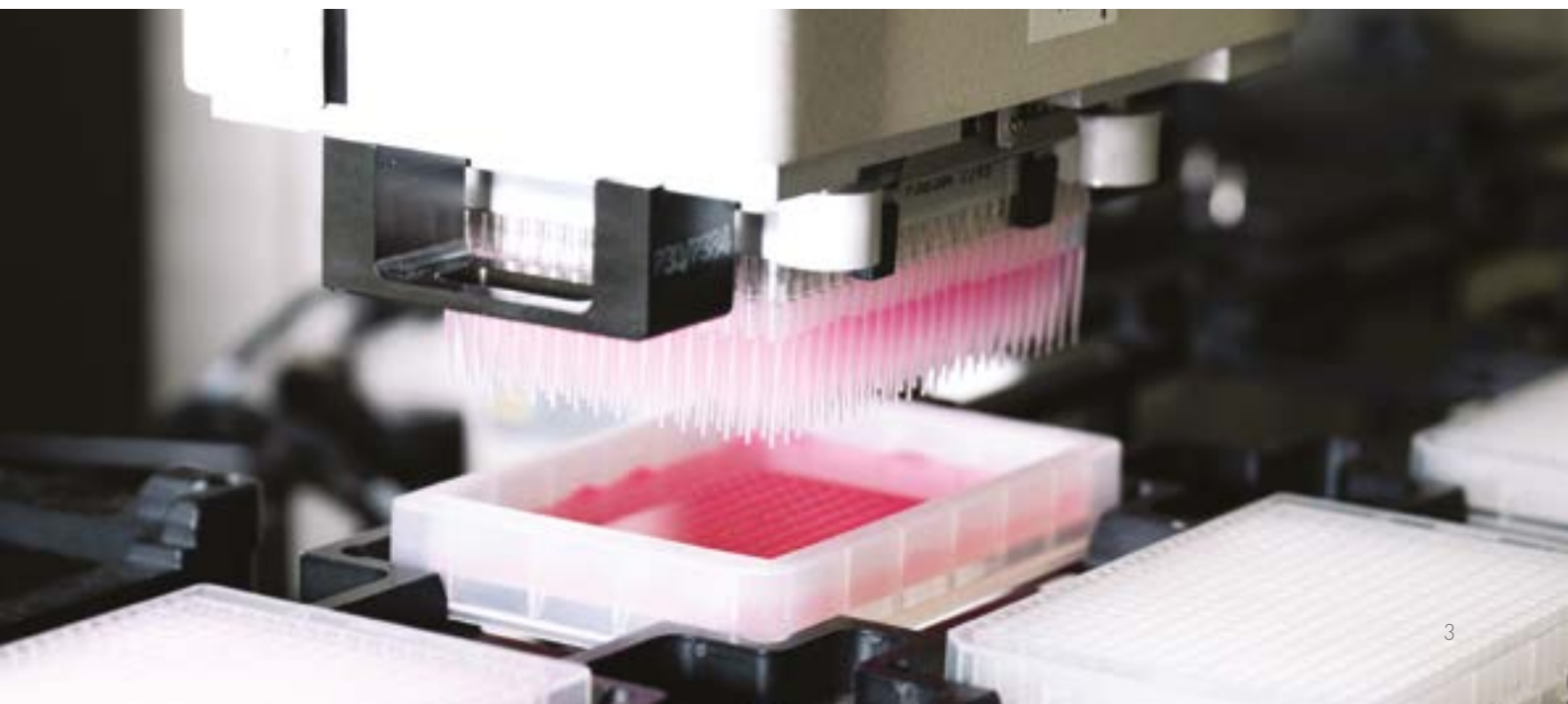


Table 1. Culture morphology

Culture format	Advantages	Disadvantages
Spheroids	<ul style="list-style-type: none"> <li>• Easy-to-use protocols</li> <li>• Scalable to different plate formats</li> <li>• HTS/HCS compatible</li> <li>• Co-culture ability</li> <li>• High reproducibility</li> </ul>	<ul style="list-style-type: none"> <li>• Simplified architecture</li> <li>• Heterogeneity of spheroid size</li> </ul>
Organoids	<ul style="list-style-type: none"> <li>• Patient specific</li> <li>• Easy-to-use</li> <li>• Scalable</li> <li>• HTS/HCS compatible</li> <li>• High reproducibility</li> </ul>	<ul style="list-style-type: none"> <li>• Lack of vasculature</li> <li>• Over complexity</li> </ul>

Table 2: HTS and HCS compatible technology

Culture format	Advantages	Disadvantages
U-Bottom ULA-plates	<ul style="list-style-type: none"> <li>• Easy-to-use</li> <li>• Non-specialized equipment required</li> <li>• Single spheroid per well</li> <li>• HTS/HCS compatible</li> </ul>	<ul style="list-style-type: none"> <li>• Low signal detection</li> <li>• Necrosis of cells in large spheroid</li> <li>• Lack of microenvironmental matrix for cells</li> <li>• Difficult to define co-culture cell composition and orientation</li> <li>• Tedious media change</li> </ul>
Scaffold/hydrogels	<ul style="list-style-type: none"> <li>• <i>In-vivo</i> relevancy</li> <li>• Defined co-culture cell composition and orientation</li> <li>• Easy-to-use</li> <li>• Scalable</li> <li>• HTS/HCS compatible</li> <li>• High reproducibility</li> <li>• Non-specialized equipment required for some animal free hydrogels</li> <li>• Multiple spheroid formation allowing higher signal detection</li> <li>• Spheroid immobilized in scaffold for easy handling</li> <li>• Easy retrieval of spheroids for further analysis using non-animal derived hydrogels</li> </ul>	<ul style="list-style-type: none"> <li>• More complex than traditional 2D approach</li> <li>• Non-uniform spheroid size</li> <li>• Specialized equipment required for animal derived hydrogel</li> <li>• Samples retrieval for further analysis difficult for animal derived hydrogels</li> <li>• Scaffold material biocompatibility and biodegradability issues</li> </ul>
Organ-on-chips	<ul style="list-style-type: none"> <li>• High level complexity</li> <li>• Cell directed orientation with other cells</li> </ul>	<ul style="list-style-type: none"> <li>• Intricate setup</li> <li>• Not routinely HTS compatible</li> </ul>

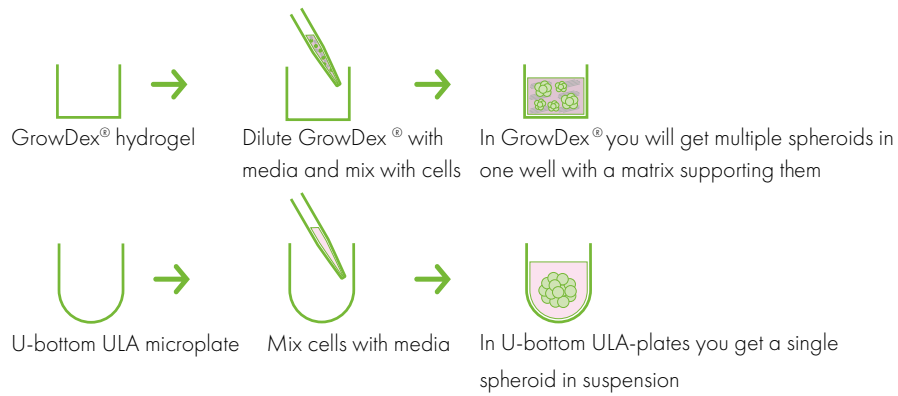


Figure 1. How do U-bottom ULA-plate cultures compare to GrowDex cultures

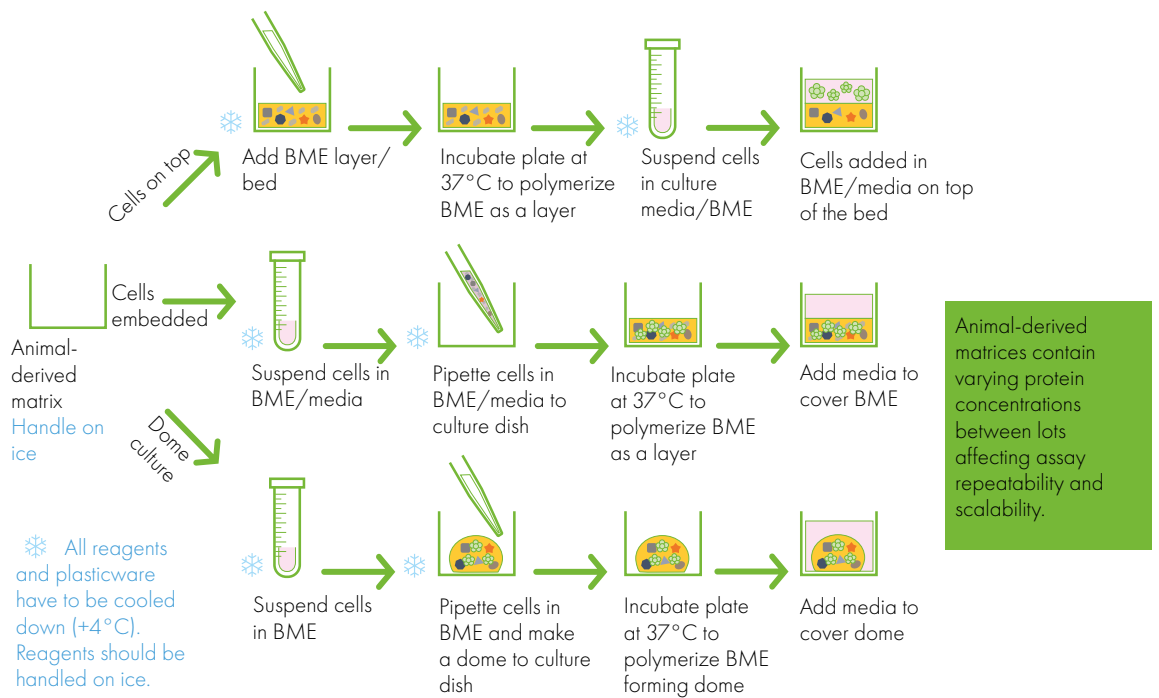


Figure 2. Culturing cells with animal-derived matrices.





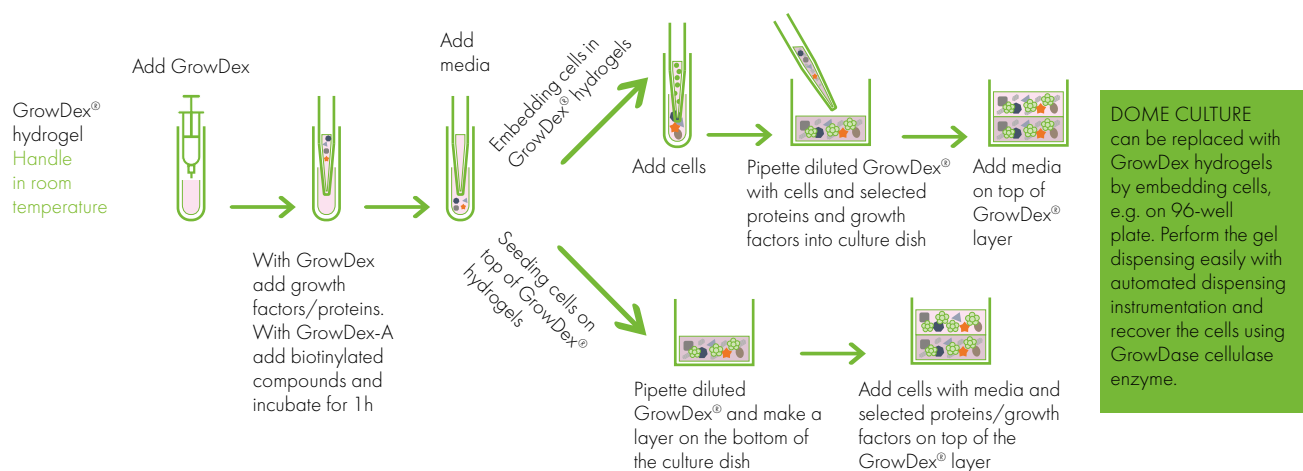


Figure 3. Culturing cells with GrowDex® hydrogels.

*“We tested using GrowDex as an alternative for animal-derived extracellular matrices in cell-based 3D high-throughput drug screening (HTS). The results demonstrated that GrowDex is compatible for use in large-scale 3D drug screens and supports long-term culture of primary patient derived tumor cell cultures.”*

*Juha K. Rantala, Ph.D., CEO, Principal Investigator at Misvik Biology*

## 1.1 The importance of automation with 3D cell culture assays

In the pharmaceutical industry HTS is extensively used and many pharmaceutical laboratories have invested in automated systems to increase the number of molecules that can be screened and to shorten the time from taking a new molecule from the labs to the clinics. For 3D cell culture models to take over 2D models in drug discovery, automation of cell culture assays is very important. Automation of 3D cell cultures assays is crucial so that the assays are reproducible and scalable. The key focus of automatization is to reduce human error, which leads to increased repeatability and reliability. Overall automation enables standardization of 3D cell culture models, so that models can be validated and approval from authorities can be obtained [2].

Currently, U-bottom Ultra-Low Adhesion plates (ULA-plates) are often used in 3D cell culture systems to generate single spheroids per well. Spheroids cultured in U-Bottom ULA-plates have gained popularity in drug screening because of their easy-to-use protocols, high-density microplate formats (e.g., 384-well and 1536-well), and compatibility with automation [3]. The U-bottom ULA-plate approach generates efficiently single spheroids, but from a biological point of view, the culture assay is missing important in vivo like properties, such as the surrounding ECM matrix that many cell types require to form in vivo -like structures and express functionality. For these applications, hydrogels have turned out to be a suitable solution. Hydrogels can be used to mimic the in vivo -like matrix by adding biological cues (e.g., ECM molecules and growth factors) and to provide physical support for the cells to grow in a 3D environment.

Rimann et. al., (2013) [4] have discussed the automation of 3D cell culture assays with hydrogels. In their article, they have concluded that animal-derived hydrogels are frequently used for 3D cell culture assays, but for HTS/HCS applications, there are additional technical optimization steps required, so that dispensing of the hydrogels is technically possible. They mention that liquid handling with animal-derived hydrogels require investments in cooled equipment, so that the hydrogel can be kept in a liquid state during the dispensing phase. The material properties of animal-derived matrices also cause challenges in gel preparation using automated systems, thus making automation very time consuming, expensive and heavy on optimization [4]. Therefore, a reproducible and automation friendly hydrogels are needed for 3D cell culture assays, so that the output capacity, accuracy, and quality of samples can be as high as in 2D HTS assays.

*“GrowDex hydrogels can be dispensed using the Certus Flex enabling excellent accuracy and reproducibility. The unique shear-thinning properties and temperature stability of GrowDex hydrogels make them ideal for high throughput applications. As the matrix stiffness of your experimental setup is crucial to cell growth, the Certus Flex can dispense different concentrations of GrowDex resulting in different densities of the final scaffold. Depending on your procedures, GrowDex allows both consecutive addition of all components (media & GrowDex and then cells in an on top layer -all simultaneously) or dispensing as premixed homogenous suspension. GrowDex hydrogels are optimally dispensed using the Certus Flex”*

-Fritz Gyger AG

## 2 Challenges scaling up 3D HTS/HCS assays using animal-derived matrices

Unfortunately, not all 3D cell culture models are compatible with HTS or HCS in a routine and cost-effective manner, so choosing the right cell culture matrix has a major impact on the efficiency and scalability of your cell culture model. The most popular animal-derived hydrogel used in cell culture is derived from extracts of Engelbreth-Holm-Swarm mouse tumours. Animal-derived hydrogels contain proteins such as collagen and laminin at unknown concentrations, as well as unknown amounts of enzymes and growth factors. Hence, it is not possible to clearly define the contents of an animal-derived hydrogel where every lot has a lot-specific protein concentration.

The properties of animal-derived matrices can possess major obstacles in scaling up the use of these hydrogels for HTS, as they are not reproducible, the cost of producing the gels are high, and there are several technical challenges handling these hydrogels [5]. These factors cause major challenges especially in drug development and HTS, where standardization and validation of models is of high interest. The material properties of animal-derived matrices and animal-free matrices have been extensively discussed in the, [“How To Transfer Your 3D Cell Culture Matrices to Animal-free GrowDex®”](#) guide. The next chapters will discuss specifically the possible challenges when animal-derived matrices are used in HTS/HCS applications.

### 2.1 Cooling down the automated liquid handling workflow

Automated liquid handling provides many benefits comparing to manual pipetting. Automated liquid handlers reduce the risk of human error and provide an ability to pipette thousands of samples per hour which saves time and money. Additionally, the instruments can pipette very precise volumes and the chance for human error is significantly less than it would be with manual liquid handling. By using an automated liquid handler, it is possible to achieve better reproducibility, accuracy, and safety.

HTS with animal-derived hydrogel requires temperature-controlled working stations, first to maintain the matrix in liquid form during the dispensing and second to heat the assay plate to induce gel polymerization, when the dispensing is complete [6]. Any errors in temperature control will lead to unexpected polymerization. In practice, HTS assays using animal-derived matrices require complex workflow setups where the liquid dispensing equipment, plasticware and consumables must be chilled to +4°C to avoid unexpected polymerization and clogging of pipette tips or tubing during dispensing. Another option for using animal-derived hydrogels in HTS/HCS is using cooled plate carriers that keep the gel in liquid state for the required time. The above-mentioned solutions are expensive, as they require complicated and thorough optimization of the HTS/HCS workflow around the used animal-derived matrix.

The material properties of animal-derived gels will lead into difficulties, when reproducible dispensing and handling of cells using automated liquid handling units is desired. The sudden polymerization and clogging of pipette tips cause variation in the dispensing results and impact the analysis of these cell assays. It has been concluded in several studies that the need to handle animal-derived hydrogels at low temperatures makes them unsuitable for common liquid handling equipment used for HTS in drug discovery [5, 7].

Therefore, hydrogels that can be used without temperature-controlled set ups would be optimal for HTS and HCS assays. GrowDex hydrogels can be handled in room temperature throughout the whole HTS/HCS workflow without any changes in the mechanical properties of the gel. As GrowDex is also a shear-thinning material, it can be handled with automated dispensing systems without any technical changes to the dispensing units, enabling accurate dispensing with little variation. This has been demonstrated with a variety of different dispensers, such as Biomek NXP, Biomek FXP, Gyger Certus Flex, Eppendorf EpMotion, Labsystems Echo 525, and Thermo Scientific Multidrop plate dispenser using 96-, 384- and 1536-well plates. GrowDex can be simply and easily incorporated into existing room temperature liquid handling workflows.

## 2.2 Autofluorescence and non-specific binding of molecules

High-content screening is based on automated digital microscopy and flow cytometry that utilizes IT-systems for data storage and analysis. The purpose of HCS is to acquire spatially (using automated microscopes) or temporally (using fluorescence measurement) resolved information on an event and to automatically quantify it. Thus, the possibility to clearly visualize cells inside of the hydrogels and stain the cells using fluorescence dyes is the basis of HCS assays. The detection of fluorescent signals, for cell viability and proliferation assays, is a key element in imaging. In 3D cell culture based HCS assays the hydrogels material properties, especially non-autofluorescence and clearness play important roles, so that automated image acquisition is possible, and the combination of automated dispensing and image acquisition can be done in one workflow.

Animal-derived hydrogels contain proteins, extracellular matrix molecules and other particles which can fluoresce and interfere with the experimental readouts. When working with animal-derived hydrogels, background fluorescence needs to be determined with a control in each experiment. As the protein concentration varies between lots, the control must be adjusted for each new animal-derived hydrogel lot acquired. It has also been reported that cells that have been collected from animal-derived hydrogels, might have residues of the matrix left that will interfere with the readouts and cause additional challenges in using automated imaging systems [8].

Cell staining dyes and immunofluorescence allows the researcher to visualise and analyse whole cells and cell components under a microscope. Since animal-derived matrices are extracted from the Engelbreth-Holm-Swarm mouse sarcoma or rat-tail collagen, primary and secondary anti-mouse antibodies must be carefully chosen and tested as they may bind to mouse proteins. This can result in non-specific binding of molecules and high background signals in imaging-based assays. It has also been reported that anti-rabbit antibodies cross react and bind to the mouse proteins in animal-derived matrices [10]. The interaction with the animal-derived hydrogels can also affect the diffusion of drug compounds, dyes and antibodies. It has been shown that different animal-derived hydrogels and synthetic scaffolds have had reduced diffusion speeds through the matrices [9].

With animal-free hydrogels such as GrowDex, adding dyes or antibody-based immunofluorescence markers can be directly added to the hydrogel, as GrowDex does not prevent the diffusion of molecules. Also, as GrowDex does not contain any animal-derived proteins, antibodies from any animal species can be used for immunofluorescence imaging and analysis. The animal-free GrowDex hydrogels contain only nanofibrillar cellulose and ultrapure water and therefore they are not autofluorescent. GrowDex is the proven solution for fluorescence spectroscopy-based imaging assays.



“Since we can degrade the GrowDex matrix using the GrowDase cellulase enzyme without disaggregating the embedded cell structures, GrowDex provides a unique flexibility in our high throughput imaging workflows. The room temperature handling also makes it easier to scale our protocols from a few wells to 1000s without seeing decrease in performance.”

Assistant Professor, Brinton Seashore-Ludlow at Karolinska institute and SciLife Lab

### 3 The cost of running 3D cell culture HTS assays

In chapter three, the estimated cost of running a HTS assay using GrowDex will be compared to running the same assay using a Engelbreth-Holm-Swarm mouse sarcoma derived hydrogel. The cost comparison is based on current retail prices for consumables, customer-based feedback, and publications (see Appendix for further details on costs).

We will present two different scenarios using the GrowDex hydrogel and the Engelbreth-Holm-Swarm mouse sarcoma derived hydrogel in a 3D cell culture HTS assays. As a reference point, also the cost of running a similar 2D cell culture HTS assay is presented. The first scenario is based on a case study conducted by Mäkelä et al. (2020) that had a 1160 compound screen, following with a validation screen of 90 compounds. The second scenario will be a hypothetical screen of a large compound library consisting of 10,000 compounds. Finally, we will explain the estimated working times for setting up assays with both gels.

#### 3.1 Scenario 1 – 1160 compound screen

In the publication Mäkelä et. al., (2020) [13], a compound library of 1160 drugs were tested for drug efficacy against ex vivo cancer organoids from a rare metastatic urachal carcinoma. The 1160 compounds were tested for a first-round screen with 3 different concentrations and a single repeat well per concentration. After the first-round screen, a second validation screen was performed with 90 compounds, using five dose concentrations and three replicates.

The estimated total cost of all consumables per culture format is shown in Figure 4 for the first-round screen. This includes the cell culture plate, culture medium and matrix if applicable. The breakdown of the costs per individual 384-well plate is shown in Figure 5. The individual component costs and experimental breakdown are listed in Table 2 as detailed in the Appendix.

Scenario 1: Estimated total cost of first-round test

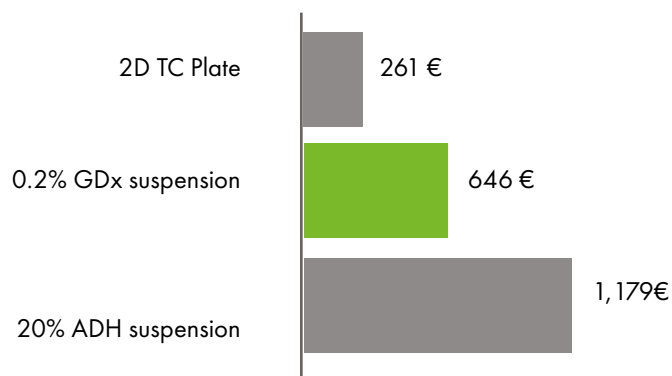


Figure 4: Estimated total cost of first-round test of 1160 compounds against a rare urachal carcinoma. GDx = GrowDex; ADH = animal derived hydrogel.

Scenario 1: Estimated cost per plate of first-round test



Figure 5: Estimated cost per plate of first-round test of 1160 compounds against a rare urachal carcinoma. GDx = GrowDex; ADH = animal derived hydrogel.

As seen in Figure 4, the estimated cost of first-round testing conducted in GrowDex would have been approximately 646€, whereas if the 3D cell culture assay was performed in the animal-derived hydrogel, the estimated cost would have nearly doubled, costing 1,179€. The comparative costs per 384 well plate is shown in Figure 5 with an estimated cost of 71€ per plate in GrowDex compared to 130€ per plate with the animal-derived hydrogel.

In the second-round screen, so called validation screen, the screen was repeated with 90 compounds with five different concentrations and three replicates. The estimated costs per format in the dose-response screen are shown in Figure 6 below.

Scenario 1: Estimated cost of dose-response test

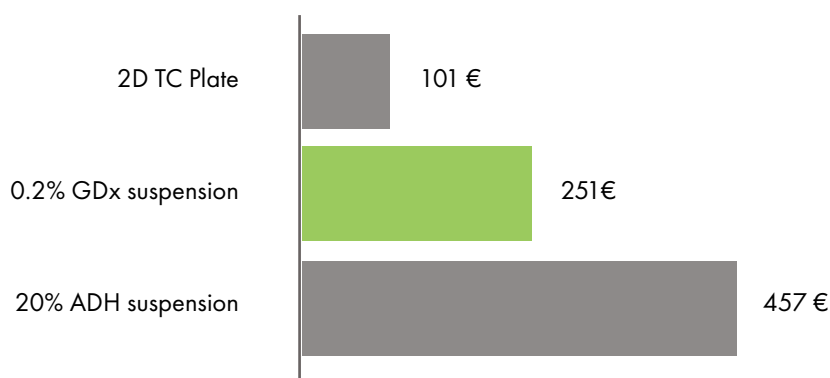


Figure 6: Estimated total cost of dose-response test of 90 compounds against a rare urachal carcinoma. GDx = GrowDex; ADH = animal derived hydrogel.

As seen in Figure 6, the estimated total cost of the dose-response test when culturing cells in GrowDex was 251€ compared to 457€ for the animal-derived hydrogel.

### 3.2 Scenario 2 – 10,000 compound screen

To illustrate the cost implications of scaling up the HTS assay, we will present this hypothetical scenario that will estimate the cost for running a HTS screen using a drug library consisting 10,000 compounds. In this scenario, the cells are cultured in suspension in a 384-well plate format, either in 0.2% GrowDex, or in 5% animal-derived hydrogel on a 50% animal-derived hydrogel bed. The culture medium is serum free (see Appendix – Medium 2). These compounds will be tested for a first-round screen with 3 different concentrations and 1 repeat well per concentration. The estimated total cost of all consumables per culture format is shown in Figure 7. The breakdown of the costs per individual 384 well plate is shown in Figure 8. The individual component costs and experimental breakdown can be seen in Table 2 as detailed in the Appendix.

Scenario 1: Estimated total cost of first-round test

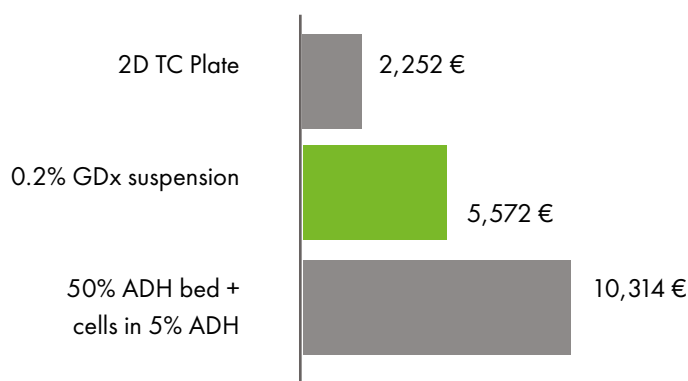


Figure 7: Estimated total cost of first-round test of 10,000 compounds.  
GDx = GrowDex; ADH = animal derived hydrogel.

Scenario 1: Estimated cost per plate of first-round test

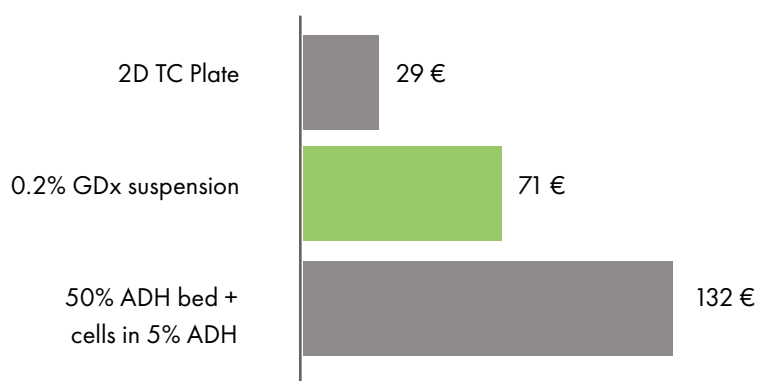


Figure 8: Estimated cost per plate of first-round test of 10,000 compounds.  
GDx = GrowDex; ADH = animal derived hydrogel.

As seen in Figure 4, the estimated total cost of the first-round testing conducted in 0.2% GrowDex would have been 5,572€, whereas if the 3D cell culture assay was performed in 5% animal-derived hydrogel on a 50% matrix bed, the total cost would raise to 10,314€. The comparative costs per 384 well plate is shown in Figure 8 with a cost of 71€ per plate with GrowDex compared to 132€ per plate in the animal-derived hydrogel.

In the dose-response validation screen, the screen was repeated with 180 compounds with 5 different concentrations using 3 repeat wells per concentration. The cost differences between the different assays are shown in Figure 9.

Scenario 1: Estimated cost of dose-response test

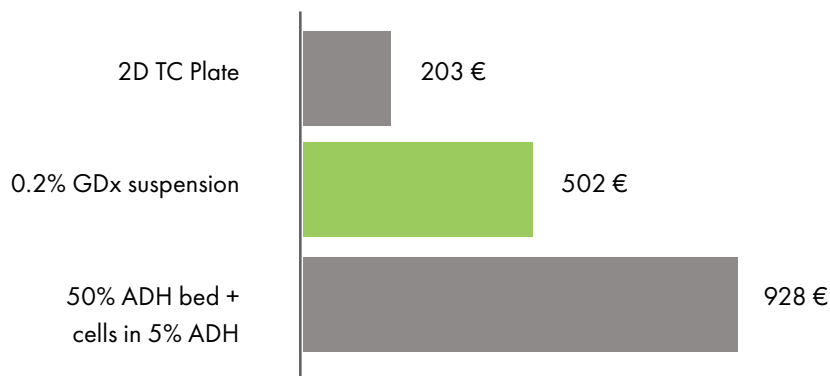


Figure 9: Estimated total cost of dose-response test of 180 compounds.

*GDx = GrowDex; ADH = animal derived hydrogel.*

As seen in Figure 9, the estimated total cost of the dose-response test when culturing cells in 0.2% GrowDex was 502 €. With the animal-derived hydrogel the cost raised to 928€.

These two scenarios illustrate the costs included in small and medium sized HTS assays. It can be clearly seen that the chosen culture format and conditions can significantly increase the cost of an initial and dose-response test. The 2D cell culture assay was in all scenarios the cheapest option. The cost of running a 3D cell culture assay is more expensive, but highly dependent on the chosen matrix. These differences become more significant, if larger compound screens would be performed.

### 3.3 Time required for setting up a screen

Finally, it is vitally important to remember that time is a crucial factor when HTS assays are conducted. To ensure a cost-effective compound screen, the time required for assay preparation should be taken in account. Significant factors affecting the overall time required to set up assays are assay setup, preparation and running the actual screen.

GrowDex can be used at room temperature and the product is ready-to-use, from the point of delivery, and therefore there is no extra time needed to prepare it for use. It is also possible to prepare a working solution of the hydrogel by diluting GrowDex prior to adding the cells that further reduces the time required for preparation. This is not possible with animal-derived hydrogels, as the stock solution needs to be separately diluted and thawed each time and cells must be added during this process to the final working solution. It is good to remember, that time is required to prepare the animal-derived matrix correctly to avoid solidification of the matrix at the wrong time [11], as well to ensure that the cells do not gravitate to the bottom of the dispensing reservoir whilst the animal-derived hydrogel is still in the liquid form [12]. The differences preparing GrowDex and the animal-derived gel have been illustrated in Figure 10.

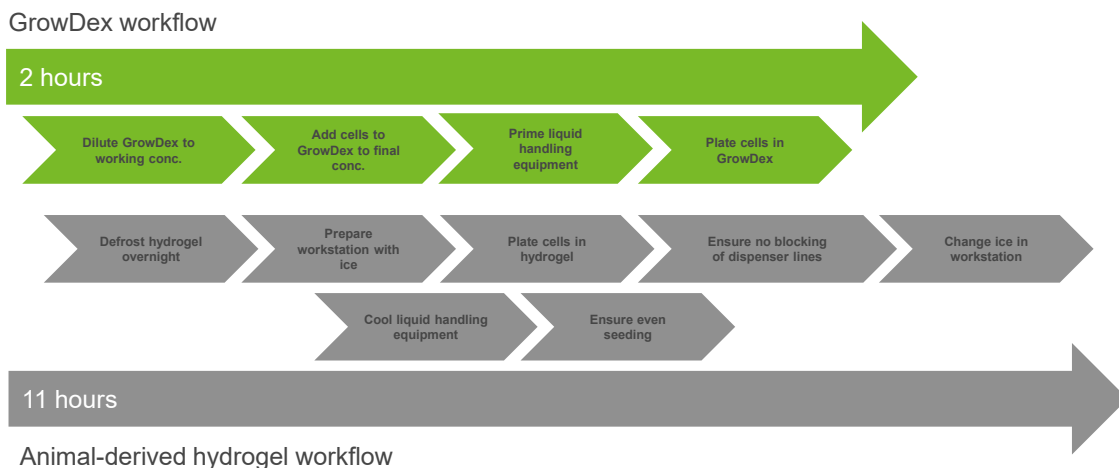


Figure 10: Workflows for HTS assay preparation when using either GrowDex or animal-derived hydrogel for 3D cell culture

An example of the time required for pre-assay setup and plating cells in 3D when working with GrowDex or the animal-derived hydrogel are illustrated in Figure 11.

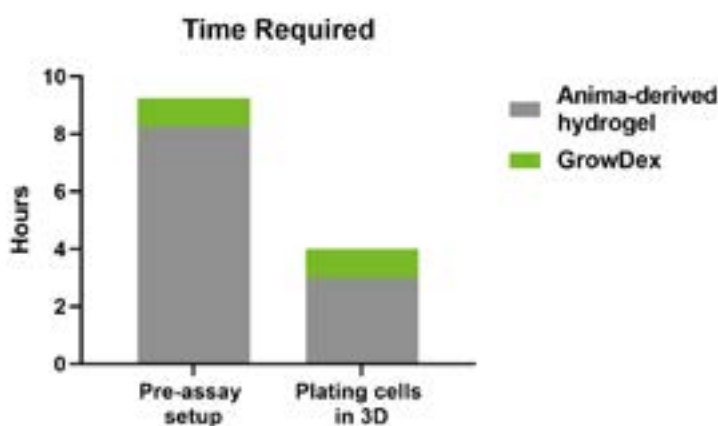


Figure 11: Estimated time required for pre-assay setup and plating cells in 3D when working with GrowDex or animal-derived hydrogel.

From Figure 11, you can see that there is a minimum of eight hours required before an assay can be performed using the animal-derived hydrogel, so that it can be defrosted to +4°C. The same step for preparing the working stock solution of GrowDex take less than one hour.

The stock solution of GrowDex can be diluted with culture medium and cells and added to a liquid handling system for plating. The diluted GrowDex with cells can be plated using the automated dispensing unit within an hour, bringing the total time of assay preparation to two hours. This step often takes longer with cells using the animal-derived matrices as shown in Figure 7, where time is required to prepare the workstation, cool the liquid handling equipment and plasticware, but also to ensure that the animal-derived hydrogel remains liquid when preparation is done. During dispensing, constant monitoring is required to ensure that pipetting the solution proceeds smoothly and the pipette tips will not clog due to unexpected polymerization. In addition, the user must be cautious about even seeding density, when mixing the animal-derived hydrogel solution with cells and to make sure sufficient cooling of used reagents and plasticware. Working with a temperature stable hydrogel shows clear time saving, when looking at assay preparation.

## 4 Conclusion

Animal-derived matrices have many challenges to be used in automated workflows for pharmacological studies of drug response. Due to their origin, collagen and hydrogels derived from extracts of Engelbreth-Holm-Swarm mouse tumours are complex scaffolds that contain many components, including components that are not known or vary based on the production lot [14, 15]. While these animal-based materials support interaction of cells with ECM proteins, due to their different composition, cells embedded into the gels can display different phenotypes [16].

From a technical perspective, animal-derived gels require heavy optimization, so that they are compatible with HTS/HCS workflows. The user must ensure that the animal-derived hydrogel remains in liquid state during dispensing with sufficient temperature control. The need for handling these hydrogels at low temperatures makes them unsuitable for common liquid handling equipment used for high-throughput screens in drug discovery [5, 7]. In addition, the polymerization through temperature changes, makes it challenging for the user to ensure that cells do not gravitate to the bottom of the culture dish, which could lead to inconsistent seeding densities and variability in setting up reproducible assays [11, 12].

In addition to the technical aspects and problems raising from the varying composition of these animal-derived hydrogels, using animal-derived hydrogels have a clear impact on the overall cost of running HTS assays. As a conclusion, the material properties of animal-derived hydrogels make setting up HTS assays technically complicated, labour intensive and expensive compared to other available solutions.

Animal-free matrices such as GrowDex hydrogels have the advantages of providing defined and tuneable material properties that allow the controlled inclusion of biochemical cues. GrowDex is produced from wood-derived nanofibrillar cellulose (NFC) and water, therefore, it does not include any traces of animal DNA or RNA. The material properties of GrowDex allows free diffusion of small and large molecules through the matrix making it possible to run large screens without interference from the matrix. The hydrogels are shear-thinning and ready-to-use, as they do not require covalent crosslinking reactions to form the gel. The hydrogels can also be pre-diluted and dispensed in room temperature conditions saving time, when preparing new assays. For HTS assays, GrowDex enables reproducible dispensing and scaling up from 96-, 384- to 1536-well plate formats. GrowDex hydrogels are the proven solution for HTS and HCS assays.





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## 5 Appendix

### 5.1 Considered costs for HTS culture

Below are components and consumables associated with 3D cell culture and HTS assay development.

Table 2: Considered costs for HTS culture

Medium 1 Components	DMEM 10 % FBS 1x Pen/Strep 1x Glutamax 1x NEAA <b>Total (500 ml of complete medium): 46 €</b>	Values used for Scenario 1
Medium 2 Components	DMEM / F-12 Ham's (1:3) 5 % FBS 1x Pen/Strep hrEGF Insulin Adenine Hydrocortisone ROCK inhibitor (Y-27632) (Xuefeng Liu et al. 2012, The Amer. J of Pathol.) <b>Total (500 ml of complete medium): 88.50 €</b>	Values used for Scenario 2
384 ULA F-bottom plate	Corning 27.80 € Revvity 26.06 €	Values used for Scenario 1 and Scenario 2
Animal-derived hydrogel Growth Factor Reduced	Animal-derived hydrogel GFR (10ml) 461 €	Values used for Scenario 1 and Scenario 2
GrowDex	GrowDex 1,5 % (10ml) 290 €	Values used for Scenario 1 and Scenario 2
Volume of matrix used/well	The presumption was made that the volume used in a 384 well plate will be ~20 µl and in a 96 well plate the volume will be ~100 µl	Values used for Scenario 1 and Scenario 2
0.2% GDx suspension	Total 11 ml; GDx stock (1.5%) 1.47 ml; Medium; 9.53 ml;	Values used for Scenario 1 and Scenario 2
20% Animal-derived hydrogel suspension	Total 11 ml; Animal-free matrix 8.8 ml; Media 2.2 ml;	Values used for Scenario 1
50% animal-free matrix bed + cells in 5% matrix	Total 9.9ml; Animal-free matrix for bed (50%) 1.95 ml; Animal-free matrix for cell suspension (5 %) 0.3 ml Media total 7.65 ml;	Values used for Scenario 2
2D TC Plate	Medium 11 ml	Values used for Scenario 1 and Scenario 2







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