GrowDex®

INSTRUCTIONS FOR USE

CONTENTS

This product is for research use only and should not be used for diagnostic or therapeutic purposes.

1. INTRODUCTION

GrowDex® has been designed to provide a support matrix for the culture of cells in a wide range of applications. It comprises of two components, nanofibrillar cellulose (NFC) and ultra-pure water. The length of the nanofibrils ranges from 200-300 µm, with a diameter in the nanometer scale varying from 5-100 nm. The product is supplied sterile, ready to use and can be utilized in a variety of cell culture applications, such as 3D spheroid or organoid formation.

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2. SAFETY INFORMATION

In accordance with current regulations (1272/2008 CLP), GrowDex is classified as non-hazardous. The product consists of micro and nanosized cellulose fibrils. The product contains 1.5% cellulose (CAS/EC number 9004-34-6 / 232-674-9) and water (CAS number 732-18-5). The nanofibrillar cellulose is isolated from the Birch tree (Betula sp.). The product is provided sterile.

The product should be handled in accordance with good laboratory (GLP) and safety practices. Use protective gloves and clothes to avoid skin exposure. If exposed wash the skin with water. Use protective laboratory eye wear to avoid contact with the eyes. In its wet state the product does not form dust. If dried however, avoid breathing the dust. Dust filters are recommended.

Description of first aid measures:

- Inhalation: Move to fresh air. Seek medical attention if symptoms appear.
- Skin contact: Rinse with water. Seek medical attention if irritation occurs.
- Eye contact: Rinse with plenty of water for several minutes. Seek medical attention if irritation occurs.
- Ingestion: Rinse mouth with plenty of water. If large quantities of the product are ingested endeavor to vomit. Seek medical attention if symptoms appear.

NOTE: For further information refer to the GrowDex® Material Safety Data Sheet.

3. PRODUCT STORAGE INSTRUCTIONS

The unopened product has a shelf life of 12 months from date of manufacture and should be stored at 4-22°C (39–72°F) and protected from light for optimum performance. Once opened it is recommended that the product is stored undiluted at 4-8°C (39-46°F) for a maximum of 3 months.

If the product has been diluted, e.g. with culture media, then it should be stored at 4-8°C (39-46°F) for a maximum of 7 days. If the media contains an unstable component, then storage time will be restricted to the shelf life of the unstable component. Please refer to the manufacturer's guidelines regarding this component.

Do not store the product below 0°C (32°F) as freezing will result in destabilization of the product rendering it unusable.

NOTE: GrowDex is supplied at a working concentration of 1.5%. It is not a concentrate.

4. PRACTICAL GUIDELINES AND RECOMMENDATIONS FOR STARTING WORK WITH GROWDEX

4.1. RECOMMENDATIONS FOR HANDLING AND PIPETTING GROWDEX

a) Low-retention pipette tips should be used to avoid GrowDex sticking to the tip.
b) A wide bore pipette tip, or one that has been cut, can help with the initial mixing step reducing to a narrower bore tip to increase mixing efficiency.
c) Aspirating and dispensing GrowDex should be performed slowly to avoid air bubbles and to ensure an accurate volume.
d) A positive-displacement pipette is useful for pipetting viscous materials like undiluted GrowDex.
e) For an exact amount of undiluted GrowDex the product can also be weighed before dilution.
f) Electric dispensing pipettes and automated dispensing systems can be used for dispensing and mixing.
g) A multi-stepper pipette or automated dispensing system is recommended for repeat dispensing of GrowDex into the well-plates for high throughput applications.

4.2. RECOMMENDATIONS WHEN USING MICROPLATES

a) Microplates containing GrowDex should be handled with care. Avoid shaking when moving the plate between locations.
b) When culturing adherent cells, the use of low-attachment micro-plates, or pre-coating with e.g. 0.4% PolyHEMA, is recommended to prevent cells attaching to the bottom of the wells.
c) GrowDex is also suitable for high throughput assays in 384 and 1536 well microplates.

4.3. RECOMMENDATIONS WHEN CHANGING MEDIA

a) When changing the media, extra care should be taken not to disturb the top of the gel. It is recommended that the well plate is slightly tilted for easier media change.
b) If loss of GrowDex occurs, then it is recommended to exchange only half the amount of media at one time.
c) The microplate can be centrifuged gently e.g. 100 x g for 5 minutes before changing the media to aid visualization of the hydrogel/media interface.

5. PROCEDURES FOR DILUTING, MIXING AND PLATING GROWDEX

The stiffness of GrowDex can be adjusted by diluting the product with e.g. cell culture media, PBS, or ultra-pure water. GrowDex concentrations of 0.2 - 1.0% are commonly used for cell culture applications. The optimal concentration will depend on the cell type being used, refer to Section 7 and the GrowDex application notes for examples and recommended assay setup details.

NOTE: GrowDex is supplied at a concentration of 1.5%, this means the product contains 1.5% cellulose and 98.5% ultra-pure water. It is not a concentrate.

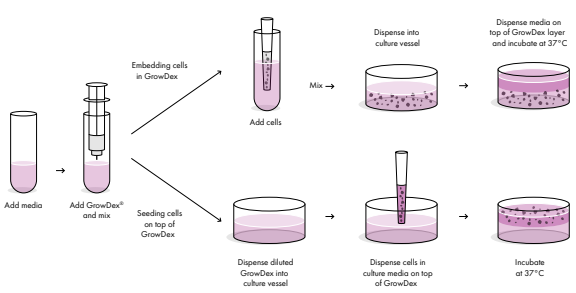

Image 1. Diluting, mixing and plating GrowDex.

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Diluting GrowDex

a) Pipette the required amount of diluent/media, without cells, into a test tube. **NOTE:** If embedding cells inside GrowDex consider the volume of the cell suspension that will be added later to ensure the correct final volume and concentration is achieved.
b) Before opening the syringe cap move the plunger gently back and forth to release it before dispensing. Dispense GrowDex directly from the syringe into the test tube containing the diluent. Graduations on the syringe indicate the volume dispensed or alternatively GrowDex may be weighed (1 ml = 1 g).
c) Mix GrowDex and diluent by first swirling the pipette tip along the wall of the tube and then by pipetting up and down for a minimum of 90 seconds. A wide bore pipette tip or one that has been cut can help with the initial mixing step. Continue until a homogenous solution is achieved by visual inspection. Increase the speed of pipetting towards the end of mixing and make sure the hydrogel flows smoothly through the pipette tip.
d) Avoid air bubble formation by keeping the pipette tip submerged in the solution throughout the mixing process.

EXAMPLE 1: Cells embedded in GrowDex
Working concentration required = 0.5%
Final volume = 1 ml

a) Pipette 567 µl culture medium into a test tube.
b) Add 333 µl GrowDex and mix by pipetting up and down until the solution is homogenous by visual inspection. (Refer to Section 5 –Procedures for diluting, mixing and plating GrowDex). Low-retention pipette tips are recommended for this procedure (Refer to Section 4 - Practical guidelines and recommendations for starting work with GrowDex).
c) Add 100 µl cell suspension to the diluted GrowDex slowly and stir carefully using the pipette tip to evenly disperse the cells.
d) Transfer 100 µl of diluted GrowDex with cells to the 96 well plate.
e) Add 100 µl of culture medium carefully on top not to disturb the GrowDex layer.
f) Incubate at 37°C.

EXAMPLE 2: Cells seeded on top of GrowDex
Working concentration required = 0.45%
Final volume = 2 ml

a) Pipette 1400 µl culture medium into a test tube.
b) Add 600 µl GrowDex and mix by pipetting up and down until the solution is homogenous by visual inspection. (Refer to Section 5 – Procedures for diluting, mixing and plating GrowDex). Low-retention pipette tips are recommended for this procedure (Refer to Section 4 - Practical guidelines and recommendations for starting work with GrowDex).
c) Transfer 100 µl of diluted GrowDex to the 96 well plate.
d) Add cells in 100 µl culture medium carefully on top of the GrowDex layer.
e) Incubate at 37°C.

6. DILUTION TABLE

Volume of GrowDex diluent, and cell suspension required for preparation of GrowDex dilution for a variety of final working concentrations.

FINAL GROWDEX CONCENTRATION	VOLUME OF GROWDEX STOCK SOLUTION (1.5 %)	VOLUME OF DILUENT	VOLUME OF CELL SUSPENSION	TOTAL VOLUME
1.0 %	800 µl	300 µl	100 µl	1200 µl
0.8 %	600 µl	425 µl	100 µl	1125 µl
0.5 %	400 µl	700 µl	100 µl	1200 µl
0.2 %	200 µl	1200 µl	100 µl	1500 µl

Please note: 'Cells seeded on top of GrowDex' procedure, add the cell suspension volume to diluent volume. Example: 0.5 % final GrowDex concentration and total volume of 1.2 ml, 400 ul of GrowDex stock + 800 ul media with cells.

7. GROWDEX CONCENTRATIONS FOR A SELECTION OF CELL-BASED ASSAYS

NEURONAL CELLS		
CELL LINE	GROWDEX CONCN	CELL SEEDING DENSITY
iPSC derived neural progenitor cells	1.0 %	500 cells / µl
hESC derived neuronal cells	1.0 %	5000 cells / µl

STEM CELLS		
CELL LINE	GROWDEX CONCN	CELL SEEDING DENSITY
Adipose tissue derived MSCs	0.2 %	1000 cells / µl
Bone marrow derived MSC's	0.5 %	2000 cells / µl
WA07, embryonic stem cells	0.5 %	5 x higher than in 2D

CANCER CELLS		
CELL LINE	GROWDEX CONCN	CELL SEEDING DENSITY
U251, glioblastoma	0.2 %	1000 cells / µl
HepG2, hepatocellular carcinoma	0.3 %	50 cells / µl
MCF7, breast adenocarcinoma	0.37 %	250 cells / µl
BT474, breast cancer	0.4 %	80 cells / µl
OVCAR8, ovarian cancer	0.8 %	100 cells / µl

HEPATIC CELLS		
CELL LINE	GROWDEX CONCN	CELL SEEDING DENSITY
HepaRG	1.0 %	1000 cells / µl
Primary hepatocytes	0.5 %	1000 cells / µl

8. ORDERING INFORMATION

CATALOGUE CODE	DESCRIPTION	QUANTITY (ml)
100 103 002	GrowDex	2.5
100 103 005	GrowDex	5.0
100 103 010	GrowDex	10.0
900 102 002	GrowDase enzyme	2.5

9. CONTACT INFORMATION

Additional information on all products and applications can be found on our website:
www.revvity.com

For a quotation or to place an order, contact us via:
www.revvity.com/contact-us

Should you have any technical questions regarding this product or its intended use please contact us via:

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