

Instructions for use

GrowDex®

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

1. SAFETY INFORMATION

GrowDex® is a nano-fibrillar cellulose (NFC) hydrogel dispersed in ultra-pure water. The product is supplied sterile and ready use and can be applied to various cell culture applications e.g. 3D spheroid formation. GrowDex® is for research use only and should not be used for diagnostic or therapeutic purposes.

In accordance with current regulations (1272/2008 CLP), this product has not been classified as dangerous. The product consists of micro and nanosized cellulose fibrils. The length of fibrils may be several micrometers while the diameter is in nanometer scale (4-100 nm). The product contains 1.5 % cellulose (CAS/EC number 9004-34-6 / 232-674-9) and water (CAS number 732-18-5). The nano-fibrillar cellulose is isolated from the birch (*Betula* sp.). The product has been autoclaved at a temperature of 120°C.

The product should be handled in accordance with good industrial hygiene 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 a 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: Wash 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 endeavour to vomit. Seek medical attention if symptoms appear.

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

2. PRODUCT STORAGE INSTRUCTIONS

The unopened product has a shelf life of 12 months from date of manufacture and should be stored in the dark at 4-22°C (39-72°F) 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 this particular component. Please refer to the manufacturers 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.

3. PRACTICAL GUIDELINES FOR STARTING WORK WITH GROWDEX®

Recommendations for pipetting GrowDex®:

- a) Low-retention pipette tips should be used to avoid GrowDex® sticking to tip.
- b) Aspirating and dispensing GrowDex® should be performed slowly to avoid air bubbles and to ensure an accurate volume.
- c) A positive-displacement pipette is useful for pipetting viscous materials like undiluted GrowDex®.
- d) For an exact amount of undiluted GrowDex® the product can be weighed before dilution.
- e) Electric dispensing pipettes can be used for mixing that may reduce user-variability.
- f) A multi-stepper pipette is recommended for repeated administration of GrowDex® into the well-plates.

Recovering GrowDex® from the vial:

- a) If GrowDex® has accumulated on the walls of the vial or water droplets are present then the vial can be centrifuged briefly to concentrate the product at the bottom for easier recovery.

Micro-plates:

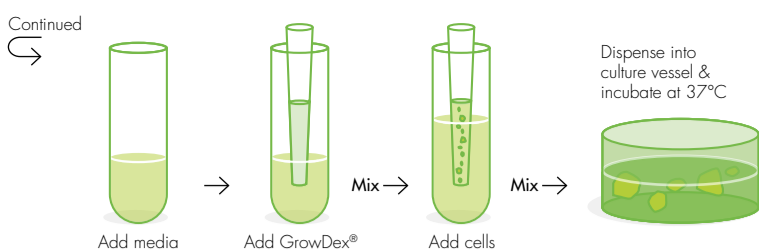
- a) Micro-plates containing GrowDex® should be handled with care. Avoid shaking when moving the plate between locations.
- b) When culturing adherent cells, the use of low-attachment micro-plates or pre-coating with e.g. PolyHEMA, is recommended to prevent cells attaching to the bottom of the wells.

Medium change:

- a) When changing the medium extra care should be taken not to disturb the top of the gel.
- b) If loss of GrowDex® occurs then it is recommended only half the amount be exchanged at one time.
- c) The microplate can be centrifuged for 5 minutes before changing the media to aid visualisation of the hydrogel/media interface.

4. RECOMMENDED PROCEDURE FOR DILUTING AND MIXING GROWDEX®

The viscosity of GrowDex® can be adjusted by diluting the product to a less viscous state. Dilution can be made 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, e.g. 0.5 % for WAO7 human embryonic stem cells and 0.8-1.0% for HepaRG and HepG2 hepatic cells. Refer to Section 7 for examples and also the GrowDex® application notes for the latest information.



The recommended procedure for the dilution and mixing of GrowDex® is as follows:

- a) Pipette the required amount of diluent, without cells, into a test tube. Take into account the volume of the cell suspension that will be added in Step (e) to ensure the correct final volume and concentration.
- b) Dispense GrowDex® directly from the syringe provided or pipette the required amount into the test tube containing the diluent. Graduations on the syringe indicate the volume dispensed or alternatively GrowDex® may be weighed.
- c) Mix the GrowDex® and diluent by pipetting up and down for a minimum of 90 seconds until a homogenous solution is achieved by visual inspection.
- d) Avoid air bubble formation by keeping the pipette tip submerged in the solution throughout the mixing process.
- e) Add the cell suspension to the test tube slowly and stir carefully with the pipette tip to avoid damaging the cells until the cells are evenly dispersed throughout the diluted GrowDex®.
- f) The diluted GrowDex®/cell mix is now ready to use.

5. EXAMPLE DILUTION PROTOCOLS FOR 3D CELL CULTURE EXPERIMENTS

EXAMPLE 1

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 4 – Recommended procedures for diluting and mixing GrowDex®). Low-retention pipette tips are recommended for this procedure (Refer to Section 3 - Practical tips for the use of 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) GrowDex® is now ready for use at a working concentration of 0.5 %.
- e) Pre-diluted GrowDex® without cells can be stored for 7 days at 4-8°C (39-46°F) if no unstable components are present in the media.

EXAMPLE 2

Working concentration required = 0.9 %
Final volume = 2 ml

- a) Pipette 600 µl culture medium into a test tube.
- b) Add 1.2 ml GrowDex® and mix by pipetting up and down until the solution is homogenous by visual inspection (Refer to Section 4 – Recommended procedures for diluting and mixing GrowDex®). Low-retention pipette tips are recommended for this procedure (Refer to Section 3 - Practical tips for the use of GrowDex®)
- c) Add 200 µl cell suspension to the diluted GrowDex® slowly and stir carefully using the pipette tip to evenly disperse the cells.
- d) GrowDex® is now ready for use at a working concentration of 0.9 %.
- e) Pre-diluted GrowDex® without cells can be stored for 7 days at 4-8°C (39-46°F) if no unstable components are present in the media.

6. DILUTION TABLE

Volume of GrowDex®, diluent and cell suspension required for the preparation of 1 ml of diluted GrowDex® for a variety of final working concentrations.

FINAL GROWDEX® CONCENTRATION	TOTAL VOLUME	VOLUME OF GROWDEX® STOCK SOLUTION (1.5 %)	DILUENT	CELL SUSPENSION
1 %	1 ml	667 µl	233 µl	100 µl
0.9 %	1 ml	600 µl	300 µl	100 µl
0.8 %	1 ml	533 µl	367 µl	100 µl
0.7 %	1 ml	467 µl	433 µl	100 µl
0.6 %	1 ml	400 µl	500 µl	100 µl
0.5 %	1 ml	333 µl	567 µl	100 µl

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

STEM CELLS			
CELL LINE	GROWDEX® CONCEN	CELL SEEDING DENSITY	COMMENTS
WA07, embryonic stem cells	0.5 %	5x higher than in 2D	Subculture every 7-12 days
IMR90-4, induced pluripotent stem cells	0.5 %	5x higher than in 2D	Subculture every 7-12 days
BM-MSC, mesenchymal stem cells	0.5 %	1000 cells / µl	65 µl GrowDex® and 65µl culture medium in each well

HEPATIC CELLS			
CELL LINE	GROWDEX® CONCEN	CELL SEEDING DENSITY	COMMENTS
HepaRG	1 %	1000 cells / µl	Use low-attachment plates
Primary hepatocytes	0.5 %	1000 cells / µl	Supplement medium with HGF and EGF

CANCER CELLS			
CELL LINE	GROWDEX® CONCEN	CELL SEEDING DENSITY	COMMENTS
HepG2, hepatocarcinoma	0.8 %	800-1000 cells / µl	Use low-attachment plates
HepG2, hepatocarcinoma	0.3 %	50 cells / µl	Use low-attachment plates Hydrogel volume 200 µl No medium on top of the hydrogel
OVCAR8, ovarian cancer	0.8 %	100 cells / µl	96 well plates pre-coated with PolyHEMA

8. ORDERING INFORMATION

CATALOGUE CODE	DESCRIPTION	QUANTITY (ml)
100 103 005	GrowDex® (supplied in syringe)	5.0
100 103 010	GrowDex® (supplied in syringe)	10.0
100 103 305	GrowDex® multipack (supplied in syringe)	3 x 5.0
900 102 002	GrowDase™ enzyme	2.5

You can order products online at: www.growdex.com

Or contact us at growdex.sales@upm.com for a quotation or to place an order.

9. CONTACT INFORMATION

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

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

EMAIL: growdex.support@upm.com

POST: UPM Biochemicals
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P.O. Box 380
00101 Helsinki
Finland

TEL: +358 (0)204 15 111
and request to speak with one of the GrowDex® team

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