

GrowDex®-A

INSTRUCTIONS FOR USE

CONTENTS

1. Introduction
2. Safety information
3. Product storage instructions
4. Practical guidelines and recommendations for working with GrowDex-A
 - 4.1. Handling and pipetting Growdex-A
 - 4.2. Microplates and media change
 - 4.2.1 Recommendations when using microplates
 - 4.2.2 Recommendations when changing media
5. Protocol for binding biotinylated compounds to GrowDex-A
6. Protocol for dilution and cell culture
7. Examples for binding, dilution and cell culture with GrowDex-A
8. Dilution table
9. Ordering information
10. Contact information

GrowDex-A is for research use only and should not be used for diagnostic or therapeutic purposes.

1. INTRODUCTION

GrowDex®-A hydrogel comprises of two components, avidin conjugated nanofibrillar cellulose and ultrapure water. Growdex-A can be used to bind different biotinylated molecules e.g. proteins, peptides, antibiotics whose presence may enhance the cell culture conditions for specific 3D cell culture applications. The product is

supplied sterile, ready to use and can be used for a variety of cell culture applications, such as 3D spheroid or organoid formation.

GrowDex-A is for research use only, not for use in diagnostic or therapeutic procedures.

2. SAFETY INFORMATION

In accordance with current regulations (1272/2008 CLP), GrowDex-A is classified as non-hazardous. The product consists of micro and nanosized cellulose fibrils. The product contains 1.0% cellulose (CAS/EC number 9004-34-6/232-674-9) and water (CAS number 732-18-5). The nanofibrillar cellulose is isolated from 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 6 months from date of manufacture and should be stored at 4–8°C (39–46°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 1 month.

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-A is supplied at a concentration of 1.0%. It is not a concentrate.

4. PRACTICAL GUIDELINES AND RECOMMENDATIONS FOR WORKING WITH GROWDEX-A

4.1. Handling and pipetting Growdex-A

- a) Before opening the GrowDex-A syringe cap move the plunger gently back and forth to release it before dispensing.
- b) Low-retention pipette tips should be used to avoid GrowDex-A sticking to the tip.
- c) A wide bore pipette tip, or one that has been cut, can help with the initial mixing step. After the mixing has been started, to increase mixing efficiency we recommend changing to a normal tip size.
- d) Aspirating and dispensing GrowDex-A should be performed slowly to avoid air bubbles and to ensure an accurate volume.

e) A positive-displacement pipette is useful for pipetting viscous materials like undiluted GrowDex-A.

f) For an exact amount of undiluted GrowDex-A the product can also be weighed before dilution.

g) Electric dispensing pipettes and automated dispensing systems can be used for dispensing and mixing.

h) A multi-stepper pipette or automated dispensing system is recommended for repeat dispensing of GrowDex-A into the well-plates for high throughput applications.

NOTE: We also recommend using low-retention tubes when working with GrowDex-A.

4.2. Microplates and media change

4.2.1. Recommendations when using microplates

- a) Microplates containing GrowDex-A should be handled with care. Avoid shaking when moving the plate between locations.
- b) When culturing adherent cells, the use of low-attachment microplates, or pre-coating with e.g. 0.4% PolyHEMA, is recommended to prevent cells attaching to the bottom of the wells.
- c) Round shaped wells or plates (96 and/or 48 well plates) are recommended to avoid hydrogel loss.

4.2.2. Recommendations when changing media

a) When changing the media, extra care should be taken not to disturb the top of the gel. The well plate can be slightly tilted for easier media change.

b) If loss of GrowDex-A 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. 100g for 5 minutes before changing the media to aid visualization of the hydrogel/media interface.

5. PROTOCOL FOR BINDING BIOTINYLATED COMPOUNDS TO GROWDEX-A

- a) Calculate the volume of stock GrowDex-A required for your experiment:

$$\text{Volume of stock GrowDex-A (ml)} = \frac{\text{Final volume (ml)} \times \text{working concentration of GrowDex-A (\%)}}{\text{Concentration of stock GrowDex-A (\%)}}$$

NOTE: The total volume of other components (biotinylated compound, cell culture media, cell suspension) is calculated as: Final volume (ml) – volume of stock GrowDex-A (ml).

- b) Calculate the amount of biotinylated compound needed (ml) as:

$$\text{Amount of biotinylated compound (ml)} = \frac{\text{Final conc. of biotinylated compound (mg/ml)} \times \text{Final volume (ml)}}{\text{Initial conc. of biotinylated compound (mg/ml)}}$$

We recommend that the biotinylated compound is added in a volume of at least 50 µl per 1 ml of GrowDex-A hydrogel, to be able to mix the reaction efficiently. If the volume of biotinylated compound to be added is very small, for example PBS can be used to dilute it.

NOTE: The molar concentration (mol/l) of biotinylated compound can be multiplied with its molar mass (g/mol) to convert it to concentration of mg/ml.

- c) Mix the stock Growdex-A with the biotinylated compound carefully by pipetting up and down to disperse throughout the gel and incubate at room temperature for 1 h.

6. PROTOCOL FOR DILUTION AND CELL CULTURE

The Growdex-A-biotinylated compound mixture will be at a slightly lower concentration than the original 1% stock due to dilution with the biotinylated compound used in step 5. This should be taken into consideration when preparing the working stock.

NOTE: We recommend using culture media without biotin. However, if using media with biotin, please select one with the lowest concentration to avoid non-specific blocking of binding sites.

a) Calculate the cell culture media volume required:

$$\text{Volume of culture media (ml)} = \text{Final volume of the assay (ml)} - \text{Volume of stock GrowDexA} \\ - \text{Volume of biotinylated compound needed (ml)} - \text{Volume of cell suspension (ml)}$$

b) Add the culture media to the test tube with the Growdex-A-biotinylated compound mixture and mix 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. Continue mixing until a homogeneous 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.

c) The cells can be either seeded on top or embedded in Growdex-A-biotinylated compound mixture, see image 1.

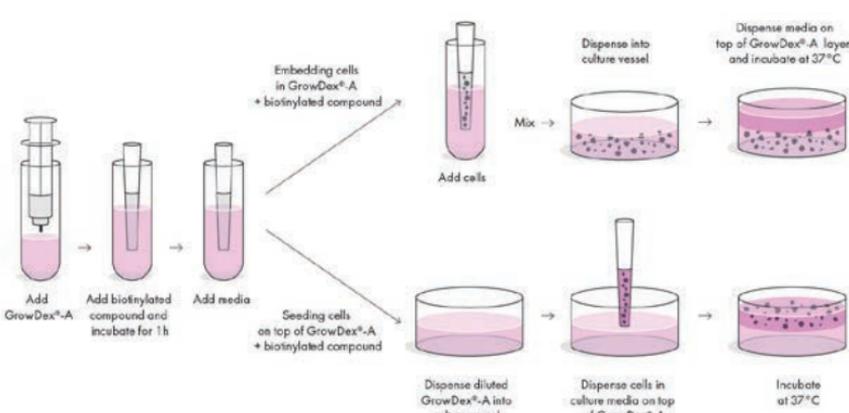


Image 1. Binding, diluting, mixing and plating of Growdex-A-biotinylated compound mixture.

7. EXAMPLES FOR BINDING, DILUTION AND CELL CULTURE WITH GROWDEX-A

EXAMPLE 1

Binding, dilution and embedding cells in Growdex-A-biotinylated compound mixture

- a) Estimate the required final volume and concentration of hydrogel. In addition, estimate the required working concentration of biotinylated compound. For example:
 Stock GrowDex-A concentration 1.0%
 GrowDex-A working concentration 0.5%
 Final volume 1 ml
 Biotinylated compound stock concentration 1 mg/ml
 Required concentration of biotinylated compound 50 µg/ml
 Cell suspension volume 100 µl

For example, for 96 well plates, we recommend using 100 µl of hydrogel per well.

NOTE: We recommend preparing some extra volume since some hydrogel might be lost when pipetting.

- b) Calculate the needed stock amount of Growdex-A:
 Volume of stock GrowDex-A (1.0%) = 1 ml * 0.5% / 1.0% = 0.5 ml

The total volume of other components (biotinylated compound, PBS/cell culture media, cell suspension) is calculated as:
 1.0 ml - 0.5 ml = 0.5 ml.

- c) Calculate the amount of biotinylated compound needed (ml) as:
 Amount of biotinylated compound (ml) = 0,05 mg/ml * 1 ml / 1 mg/ml = 0.05 ml

When the biotinylated compound stock concentration is 1 mg/ml, the volume of biotinylated compound needed is 0.05 ml = 50 µl.

- d) When the volume of cell suspension needed for the experiment is taken into consideration, the volume of culture media to be added to the functionalized gel can be calculated by following equation:

$$\text{Volume of culture media (ml)} = 1.0 \text{ ml} - 0.5 \text{ ml} - 0.05 \text{ ml} - 0.1 \text{ ml} = 0.35 \text{ ml}$$

- e) Add 500 µl of stock GrowDex-A into a test tube.
 f) Add 50 µl of biotinylated compound and mix by gently pipetting up and down until homogenous mixture is obtained. Incubate at room temperature for one hour.
 g) Add 350 µl of cell culture media and mix until homogenous suspension is achieved.
 h) Add 100 µl of cell suspension and mix gently.
 i) Transfer 100 µl of sample per well on 96 well plate.
 j) Add 100 µl of culture medium carefully on top not to disturb the functionalized GrowDex-A layer.
 k) Incubate at 37°C.

EXAMPLE 2

Seeding cells on top of Growdex-A-biotinylated compound mixture

Stock GrowDex-A concentration 1.0%
 Working concentration required = 0.45%
 Final volume = 2 ml

- a) Add 900 µl of stock GrowDex-A into a test tube.
 b) Add 100 µl of biotinylated compound and mix by gently pipetting up and down until homogenous mixture is obtained. Incubate at room temperature for at least one hour.

- c) Add 1000 µl of culture medium and mix by pipetting up and down until the solution is homogenous by visual inspection. Low-retention pipette tips are recommended for this procedure (Refer to Section 4 – Practical guidelines and recommendations for working with GrowDex-A)
 d) Transfer 100 µl of diluted GrowDex-A-biotinylated compound mixture to the 96 well plate.
 e) Add cells in 100 µl culture medium carefully on top of the diluted GrowDex-A-biotinylated compound mixture layer.
 f) Incubate at 37°C.

8. DILUTION TABLE

Table 1 displays the volume of stock Growdex-A, biotinylated compound, culture media, and cell suspension required for the preparation of 1 ml of Growdex-A-biotinylated compound mixture for a variety of final working concentrations.

Table 1. Example of dilutions for Growdex-A if biotinylated compound stock concentration is 1 mg/ml and working concentration 50 µg/ml.

FINAL GROWDEX-A CONCENTRATION	TOTAL VOLUME	VOLUME OF GROWDEX-A STOCK SOLUTION (1.0%)	BIOTINYLATED COMPOUND VOLUME	CULTURE MEDIA	CELL SUSPENSION
0.7%	1 ml	700 µl	50 µl	150 µl	100 µl
0.6%	1 ml	600 µl	50 µl	250 µl	100 µl
0.5%	1 ml	500 µl	50 µl	350 µl	100 µl
0.4%	1 ml	400 µl	50 µl	450 µl	100 µl
0.3%	1 ml	300 µl	50 µl	550 µl	100 µl
0.2%	1 ml	200 µl	50 µl	650 µl	100 µl
0.1%	1 ml	100 µl	50 µl	750 µl	100 µl

NOTE: For more information on biotinylated compounds please see our application notes.

9. ORDERING INFORMATION

CATALOGUE CODE	DESCRIPTION	QUANTITY (ml)
300 103 005	GrowDex-A	5.0
300 103 010	GrowDex-A	10.0
300 103 305	GrowDex-A multipack	3 x 5.0

You can order products online at: www.upmbiomedicals.com/store

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

10. CONTACT INFORMATION

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

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

EMAIL: biomedicals.support@upm.com

POST: UPM Biomedicals
 Alvar Aallon katu 1
 P.O. Box 380
 00101 Helsinki
 Finland

TEL: +358 (0)204 15 111

and request to speak with someone of the Biomedicals team

FAX: +358 (0)204 15 110

GrowDex®-A