UPMBIOFORE
BEYOND FOSSILS

GrowDase™

INSTRUCTIONS FOR USE

CONTENTS

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

1. INTRODUCTION

GrowDase™ is a purified mixture of cellulase enzymes (10 mg/ml) that has been specifically developed to breakdown the nanofibrillar cellulose present in UPM's GrowDex® to soluble glucose. The process is simple, enzyme is mixed with the sample and incubated at 37°C until the GrowDex has been reduced to non-toxic sugars. Enzymatic removal allows cells to be recovered from the matrix efficiently, retain their 3D structure, e.g. spheroid or organoid in-tact and does not exhibit any adverse effect on cell viability or functionality. The amount of GrowDase required for cell recovery is dependent on the amount of GrowDex (cellulose) present in the sample. One GrowDase vial (2.5 ml) can degrade 5 ml of GrowDex. Recovery of cells, organoids or spheroids for downstream processing once a live cell assay has been concluded can be advantageous for many cell-based studies. Additional data relating to gene or protein expression can be generated or cells used for further experimental work such as in multi-dose studies.

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

2. SAFETY INFORMATION

GrowDase consists of a cellulase enzyme mixture in aqueous sodium acetate buffer solution, pH 5. The product is sterile and intended for the degradation of GrowDex. GrowDase is for research use only, not for diagnostic or therapeutic use.

In accordance with current regulations (1272/2008 CLP), this substance has been classified as non-hazardous. The product contains up to 10 mg/ml enzymes and has been sterile filtered.

The product should be handled in accordance with good laboratory practices (GLP). 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.

Hazard Statements:
EUH208 Contains enzymes. May produce an allergic reaction.

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.

Most important symptoms and effects, both acute and delayed

- Inhalation: May produce an allergic reaction.
- Ingestion: No symptoms or effects known.
- Skin contact: No symptoms or effects known.
- Eye contact: No symptoms or effects known.

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

3. PRODUCT STORAGE INSTRUCTIONS

The 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 at 4-8°C (39-46°F) for a maximum of 3 months.

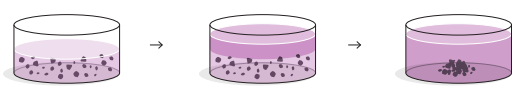
If the product has been diluted, e.g. with culture media, then it should be used immediately. Any leftover diluted enzyme should be discarded.

4. GROWDASE IS USED FOR THE ENZYMATIC DEGRADATION OF GROWDEX

GrowDase is a purified mixture of cellulase enzymes that has been specifically developed to reduce the nanocellulose fibrils present in GrowDex to soluble glucose. The process is simple, the enzyme is mixed with the sample and incubated at 37°C until the GrowDex has been fully reduced to glucose. Enzymatic removal allows cells to be recovered from the matrix efficiently, retain their 3D structure, e.g. spheroid or organoid, intact and without adverse effects on cell functionality.

The amount of GrowDase required for cell recovery is dependent on the amount of GrowDex hydrogel (cellulose) present in the sample.

It is recommended that GrowDase is used at a working concentration of 300 µg/mg of GrowDex. An equal volume of working concentration GrowDase to GrowDex/cell matrix volume present in the sample should be used, i.e. if 100 µl of GrowDex/cell matrix is present in the sample then 100 µl of GrowDase (300 µg/mg) should be added to that sample well.



Cells cultured in GrowDex → GrowDase added, sample incubated at 37°C → GrowDex degraded to glucose, cells are in solution

5. PROCEDURE FOR REMOVING GROWDEX HYDROGEL BY ENZYMATIC DEGRADATION

a) Calculate the amount of cellulose present in the sample well using the following equation.

NOTE: 100 µl of 1% GrowDex = 1 mg cellulose .

Sample well volume (µl) x % GrowDex concentration / 100% = cellulose/sample well (mg)

b) Calculate the amount enzyme needed to degrade the cellulose in the sample well using the following equation.

NOTE: 300 µg GrowDase enzyme is required to degrade 1 mg of cellulose.

Amount of cellulose/sample well (mg) x 300 µg/mg = Amount of GrowDase enzyme (µg)

c) Calculate the volume of GrowDase stock solution needed using the following equation:

GrowDase amount (µg) / GrowDase stock concentration (10 µg/µl) = Volume of GrowDase stock solution (µl)

d) Prepare the working concentration of GrowDase by diluting the stock solution with culture media. The volume of GrowDase working solution is recommended to be the same as the volume of GrowDex in the well:

GrowDex volume in well (µl) – Volume (µl) of GrowDase stock solution (10 mg/ml) = Volume of culture media for dilution (µl)

e) Pipette the diluted GrowDase onto the top of the sample in the microplate.

f) Incubate the plate at 37°C for a minimum of 8 hours until the hydrogel has fully degraded.

g) Recover the cells from the well using standard techniques.

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6. EXAMPLE EXPERIMENTAL PROCEDURE

SAMPLE: 80 µl of 0.9% GrowDex/cell mix per well in a 96-well microplate

MATERIALS

- GrowDase enzyme, 10 mg/ml (10 µg/µl)
- Cell culture medium

METHOD

a) a) Amount of cellulose present in the sample: *80 µl x 0.9%/100% = 0.72 mg cellulose/sample well*

b) Amount of GrowDase needed to degrade the cellulose in the sample: *0.72 mg x 300 µg/mg = 216 µg GrowDase enzyme*

c) Volume of GrowDase stock solution needed: *216 µl / 10 µg / µl = 21.6 µl GrowDase enzyme stock solution*

d) Prepare the working concentration of GrowDase by diluting the stock solution (10 mg/ml) with culture media: *80 µl - 21.6 µl = 58.4 µl culture media for dilution*

e) Pipette the diluted GrowDase onto the top of the sample in the microplate.

f) Incubate the plate at 37°C until the hydrogel has fully degraded.

g) Recover the cells from the well using standard techniques.

7. DILUTION TABLE

Volumes of GrowDase enzyme and cell culture medium required for the preparation of 100 µl of GrowDase working solution (300 µg/mg) for the degradation of 100 µl of GrowDex.

GROWDEX CONCENTRATION IN 100 µL OF SAMPLE	AMOUNT OF GROWDASE ENZYME NEEDED	VOLUME OF GROWDASE ENZYME STOCK SOLUTION (10 MG/ML)	VOLUME OF CELL CULTURE MEDIUM
1.0%	300 µg	30 µl	70 µl
0.9%	270 µg	27 µl	73 µl
0.8%	240 µg	24 µl	76 µl
0.7%	210 µg	21 µl	79 µl
0.6%	180 µg	18 µl	82 µl
0.5%	150 µg	15 µl	85 µl
0.4%	120 µg	12 µl	88 µl
0.3%	90 µg	9 µl	91 µl
0.2%	60 µg	6 µl	94 µl

8. ORDERING INFORMATION

CATALOGUE CODE	DESCRIPTION	QUANTITY (ml)
900 102 001	GrowDase enzyme	1.25
900 102 002	GrowDase enzyme	2.5
100 103 905	GrowDex + GrowDase combo pack	5.0 + 2.5

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.

9. CONTACT INFORMATION

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

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GrowDase™