GrowDex®

Application Note 4

UPMBIOFORE BEYOND FOSSILS

Guidelines for recovering cells and spheroids from GrowDex® using GrowDase™ enzyme

GROWDASE IS USED FOR THE ENZYMATIC DEGRADATION OF GROWDEX

GrowDase™ is a purified mixture of cellulase enzymes that have been specifically prepared to degrade the nanocellulose fibrils present in GrowDex®, reducing them to soluble glucose. This simple enzymatic removal procedure allows cells to be recovered from the matrix efficiently, without adverse effects and retain any 3D structure such as a spheroid or organoid fully intact. The degradation process is simple; enzyme is mixed with cell culture media and incubated with GrowDex at 37°C until the hydrogel has been dissolved away (Fig. 1).

The amount of GrowDase required for cell recovery is dependent on the amount of GrowDex (cellulose) present in the experimental well. It is recommended that GrowDase is used at a working concentration of 300 µg/mg (µg enzyme/mg cellulose). An equal volume of working concentration GrowDase to GrowDex/cell matrix volume present in the experimental well should be used, i.e. if 100 µL of GrowDex/cell matrix is present in the experimental well then 100 µl of GrowDase should be added to that well.

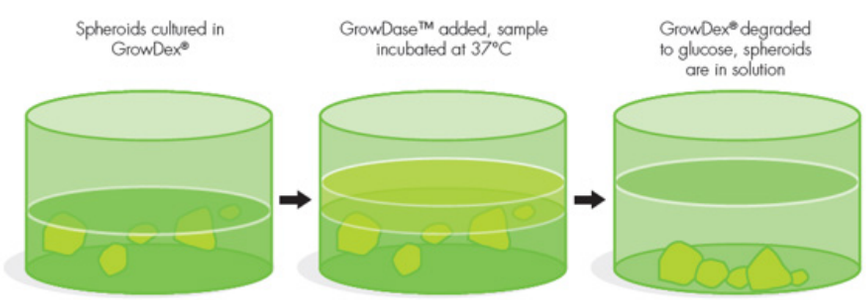

Figure 1. Schematic presentation of GrowDex degradation with GrowDase

PROCEDURE FOR REMOVING GROWDEX BY ENZYMATIC DEGRADATION

1. Calculate the amount of cellulose present in the sample well using the following equation. NOTE: 100 µL of 1% GrowDex contains 1 mg cellulose.

$$\textit{Sample well volume}\ (\mu L) \times \textit{GrowDex concentration}/100 = mg\ \textit{GrowDex/sample well}$$

2. Prepare the working concentration of GrowDase by diluting the stock solution with culture media.
3. Pipette the diluted GrowDase onto the top of the sample in the microplate.
4. Incubate the plate at 37°C until the hydrogel has fully degraded.
5. Recover the cells from the well using standard techniques.

EXAMPLE EXPERIMENTAL PROCEDURE

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

1. Amount of GrowDex present in the sample:
   80 µL x 0.9 / 100 = 0.72 mg GrowDex/ sample well.

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

3. Volume of GrowDase stock solution (10 mg/mL) needed:
   216 µg / 10 µg/µL = 21.6 µL GrowDase enzyme stock solution.

4. Prepare the working concentration of GrowDase by diluting the stock solution with culture media:
   80 µL - 21.6 µL = 58.4 µL culture media for dilution.

5. Pipette the diluted GrowDase onto the top of the sample, incubate at 37°C until the hydrogel has fully degraded and recover cells from the well using standard techniques.

DILUTION TABLE

Volumes of GrowDase enzyme stock solution (10 mg/mL) and cell culture medium required for the preparation of 100 µL of 300 µg/mg enzyme working solution for the degradation of 100 µL GrowDex in different concentrations from 0.3% to 1%.

GROWDEX CONC IN 100 µL OF SAMPLE	AMOUNT OF GROWDASE NEEDED	VOLUME OF GROWDASE STOCK SOLUTION (10 MG/ML)	VOLUME OF CULTURE MEDIA
1%	300 µl	30 µl	70 µl
0.9%	270 µl	27 µl	73 µl
0.8%	240 µl	24 µl	76 µl
0.7%	210 µl	21 µl	79 µl
0.6%	180 µl	18 µl	82 µl
0.5%	150 µl	15 µl	85 µl
0.4%	120 µl	12 µl	88 µl
0.3%	90 µl	9 µl	91 µl

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

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

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