GrowDex®-A

Application Note 1

UPMBIOFORE BEYOND FOSSILS

Recommended Procedure For Binding Biotinylated Proteins to GrowDex®-A

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GrowDex®-A is avidin-conjugated nanofibrillar cellulose hydrogel suitable for use in a variety of 3D cell culture applications. Avidin is able to bind to biotin with high affinity and specificity and this phenomenon has been exploited in many technologies e.g. ELISA assays. GrowDex-A can be customised by binding different biotinylated molecules e.g. proteins or peptides, to create a cell specific matrix for 3D cell-based assays (Fig. 1).

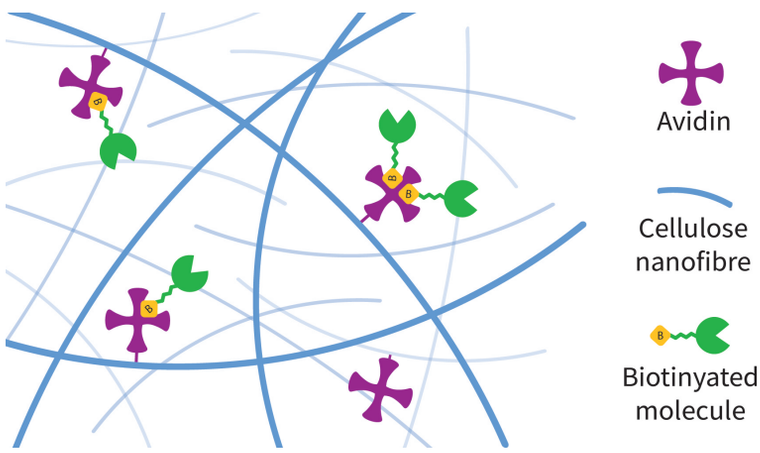

Figure 1. Illustration of binding biotinylated molecules to GrowDex-A hydrogel

PROTOCOL FOR BINDING BIOTINYLATED COMPOUNDS TO GROWDEX-A

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

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

B. Before opening the GrowDex-A syringe cap, move the plunger slightly back and forth to release it before dispensing.

C. Dispense GrowDex-A directly from the syringe provided or pipette the required amount into the test tube. Graduations on the syringe indicate the volume dispensed or alternatively GrowDex-A may be weighed.

D. Calculate the volume of biotinylated compound needed (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 for diluting it.

$$Volume\ of\ biotinylated\ compound\ (ml)\ = \frac{Final\ conc.of\ biotinylated\ compound\ (mg/ml)\ x\ Final\ volume\ (ml)}{Initial\ conc.of\ biotinylated\ compound\ (mg/ml)}$$

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

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. If you have different media that contain biotin, we recommend selecting the media which contains the least amount of biotin.

A. Calculate the volume of cell culture media required:

$$\begin{aligned} &Volume\ of\ culture\ media\ (ml) \\ &= Final\ volume\ of\ the\ assay\ (ml) - Volume\ of\ stock\ GrowDex - A \\ &- Volume\ of\ biotinylated\ compound\ (ml) - Volume\ of\ cell\ suspension\ (ml) \end{aligned}$$

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 Fig. 2.

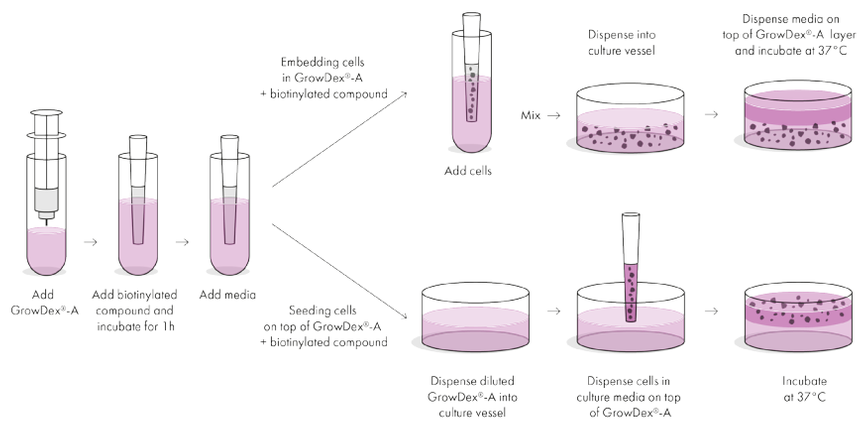

Figure 2. Binding, diluting, mixing, and plating of GrowDex-A-biotinylated compound mixture.

EXAMPLE FOR BINDING, DILUTION AND EMBEDDED CELL CULTURE WITH GROWDEX-A

- 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

NOTE: For example, for 96 well plates, we recommend using 100 µl of hydrogel per well. We recommend preparing some extra volume since some hydrogel might be lost when pipetting.

A. 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.

B. 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.

C. 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:
Volume of culture media (ml) = 1.0 ml – 0.5 ml – 0.05 ml – 0.1 ml = 0.35 ml

D. Add 500 µl of stock GrowDex-A into a test tube.
E. 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.
F. Add 350 µl of cell culture media and mix until homogenous suspension is achieved.
G. Add 100 µl of cell suspension and mix gently.
H. Transfer 100 µl of sample per well on 96 well plate.
I. Add 100 µl of culture medium carefully on top not to disturb the GrowDex-A layer.
J. Incubate at 37°C.

DILUTION TABLE

Table 1. Volumes 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.

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

ORDERING INFORMATION

CATALOGUE CODE	DESCRIPTION	QUANTITY (ml)
300 103 005	GrowDex-A	5.0
300 103 010	GrowDex-A	10.0
300 103 305	1ml	3 x 5.0

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