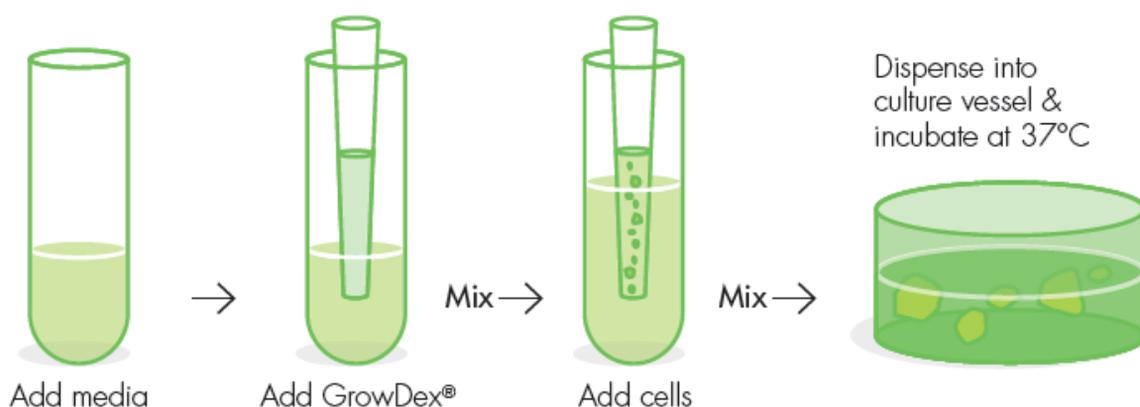


Guidelines for using GrowDex[®] in cell-based assays

RECOMMENDED PROCEDURE FOR DILUTING AND MIXING GROWDEX

GrowDex can easily be diluted with e.g. cell culture media, PBS, or ultra-pure water for use in cell-based assays. 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 WA07 human embryonic stem cells and 0.8-1.0% for HepaRG and HepG2 hepatic cells. Refer to 'GrowDex Instructions for use' for more examples and also to other GrowDex application notes for detailed information.

The viscosity of GrowDex is adjusted by diluting the product to a less viscous state.



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

1. 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 5 to ensure the correct final volume and concentration.
2. 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.
3. 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.
4. Avoid air bubble formation by keeping the pipette tip submerged in the solution throughout the mixing process.
5. 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.
6. The diluted GrowDex/cell mix is now ready to use.

EXAMPLE DILUTION PROTOCOL FOR 3D CELL CULTURE EXPERIMENTS

Working concentration required = 0.5 % / Final volume = 1 mL

1. Calculate the needed amounts of stock GrowDex (1.5%) and cell culture medium.
NOTE: Take into account the volume in which the cells are seeded into GrowDex

$$\text{Volume of stock GrowDex (1.5\%)} = \frac{\text{Final volume of assay} \times \text{required GrowDex concentration (\%)}}{1.5\%}$$

$$\text{Volume of culture media} = \text{Final volume of assay} - \text{Volume of stock GrowDex} - \text{Volume of cell suspension}$$

2. Pipette 567 µL culture medium into a test tube.
3. Add 333 µL GrowDex and mix by pipetting up until the solution is homogenous by visual inspection.
 - A. Low-retention pipette tips are recommended for this procedure. More practical tips for the use of GrowDex can be found in Section 3 in 'GrowDex Instructions for use'.
 - B. Pre-diluted GrowDex without cells can be stored for 7 days at 2-8°C if no unstable components are present in the media.
4. Add 100 µL cell suspension to the diluted GrowDex slowly and stir carefully using the pipette tip to evenly disperse the cells.
5. GrowDex is ready for use at a working concentration of 0.5 %.

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 %)	VOLUME OF DILUENT	VOLUME OF 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

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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