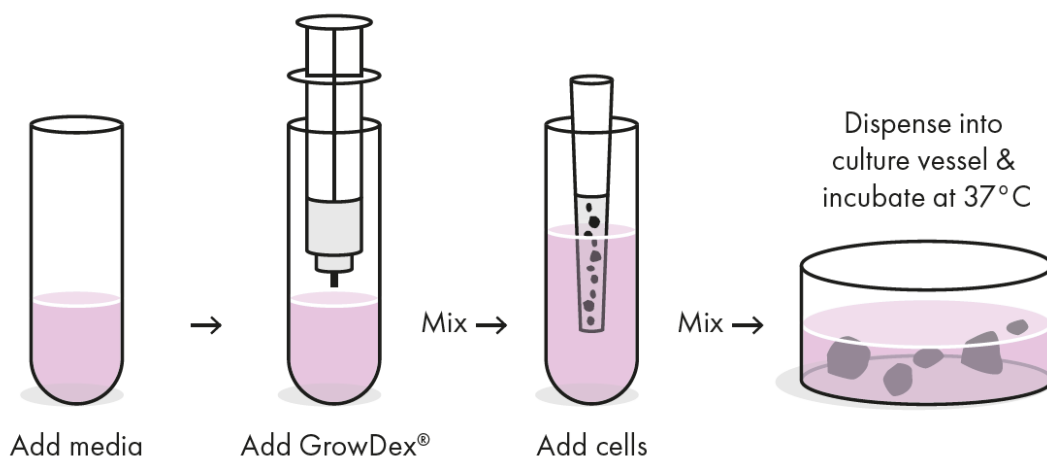


Recommended Procedure For Diluting and Mixing GrowDex[®]-T

GrowDex[®]-T can easily be diluted with e.g. cell culture media, PBS, or ultra-pure water for use in cell-based assays. Concentrations of 0.2-0.9% are commonly used for cell culture applications. The optimal concentration will depend on the cell type being used, refer to 'GrowDex-T Instructions for use' and other GrowDex-T application notes for examples and recommended assay setup details.

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



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

1. Pipette the required amount of diluent, without cells, into a test tube. **NOTE:** Take into account the volume of the cell suspension that will be added in Step (e) to ensure the correct final volume and concentration.
2. Before opening the GrowDex-T syringe cap, move the plunger slightly back and forth to release it before dispensing. Dispense GrowDex-T 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-T may be weighed.
3. Mix GrowDex-T and diluent 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 or continue until a homogenous 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. Using a wider bore pipette tip or one that has been cut can help with the initial mixing step. Low-retention pipette tips are recommended for this procedure to avoid hydrogel adhering to the tips.
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-T.
6. The diluted GrowDex-T cell mix is now ready to use.



EXAMPLE DILUTION PROTOCOL FOR 3D CELL CULTURE EXPERIMENTS

Working concentration required = 0.25% / Final volume = 1 ml

1. Calculate the needed amounts of stock GrowDex-T (1.0%) and cell culture medium. NOTE: Take into account the volume in which the cells are seeded into GrowDex-T

Volume of stock GrowDex – T (1.0%) =
$$\frac{\text{Final volume of assay} \times \text{required GrowDex – T concentration (\%)}}{1.0 \%}$$

Volume of culture media = Final volume of assay – Volume of stock GrowDex – Volume of cell suspension

2. Pipette 650 µl culture medium into a test tube.
3. Add 250 µl GrowDex-T and mix by swirling the pipette tip against the tube wall and then by pipetting up and down until the solution is homogenous by visual inspection.
4. Add 100 µl cell suspension to the diluted GrowDex-T slowly and stir carefully using the pipette tip to evenly disperse the cells.
5. GrowDex-T is now ready for use at a working concentration of 0.25%.
6. Pre-diluted GrowDex-T without cells can be stored for 7 days at 4-8°C (39-46°F) if no unstable components are present in the media.

DILUTION TABLE

Volume of GrowDex-T, diluent and cell suspension required for the preparation of 1 ml of diluted GrowDex-T for a variety of final working concentrations.

FINAL GROWDEX®-T CONCENTRATION	TOTAL VOLUME	VOLUME OF GROWDEX®-T STOCK SOLUTION (1.0%)	DILUENT	CELL SUSPENSION
0.9%	1 ml	900 µl	0 µl	100 µl
0.8%	1 ml	800 µl	100 µl	100 µl
0.7%	1 ml	700 µl	200 µl	100 µl
0.6%	1 ml	600 µl	300 µl	100 µl
0.5%	1 ml	500 µl	400 µl	100 µl
0.4%	1 ml	400 µl	500 µl	100 µl
0.3%	1 ml	300 µl	600 µl	100 µl
0.2%	1 ml	200 µl	700 µl	100 µl

ORDERING INFORMATION

CATALOGUE CODE	DESCRIPTION	QUANTITY (ml)
200 103 002	GrowDex®-T	2.5
200 103 005	GrowDex®-T	5.0
200 103 010	GrowDex®-T	10.0
200 103 305	GrowDex®-T multipack	3 x 5.0
200 103 905	GrowDex®-T 5 ml + GrowDase™ combo pack	5.0 + 2.5
900 102 002	GrowDase™ Enzyme	2.5

