Corning® 3D Clear Tissue Clearing Reagent

Quick Start Guide

READ THIS BEFORE WORKING WITH CORNING 3D CLEAR TISSUE CLEARING REAGENT

Corning 3D Clear is designed to simplify tissue clearing and visualization, but there are some best practices that will help optimize the process. Please review this Quick Start Guide which outlines considerations and preparation steps you should follow before you begin to label, clear, and image tissue samples.

Label Optimization → Permeabilization → Labeling → Clearing → Imaging

To maximize your results using Corning 3D Clear, follow the steps below.

Fluorescent Protein	Nuclear and Viability Stains
Unless combined with immunolabeling, there is no need to perform permeabilization or labeling steps.	
Proceed directly to clearing, and perform dehydration with ethanol at 4°C (see reverse side).	Apply stain as directed by manufacturer, fix tissue, and proceed directly to dehydration and clearing (see reverse side).

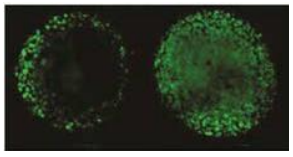
Immunolabeling

Optimizing the antibody dilution is a critical step to successful tissue clearing. We recommend taking a few days to optimize your tissue staining, which overall will save you time and effort moving forward.

1. Optimizing dilution and duration: Use at least six *in vitro* models to explore dilutions ranging from 1:500 to 1:50 and durations from 30 minutes to 12 hours.
2. Image using a confocal microscope and look for even staining in Z sections.
3. If antibody concentration is too low, there will be low signal-to-noise ratio. If it's too high, the outer layers will "shadow" the inner layers due to the absorbency in the outer layers.
4. The antibody solution should completely cover the tissue of interest.

Optimization at a Glance

Try conditions on a single culture → Examine uniformity and depth



Non-uniform Labeling | Uniform Labeling

Permeabilization

		3D Cell Culture
1X	PBS	15 min.
1X	MeOH	15 min.
1X	20% DMSO/MeOH	15 min.
1X	MeOH	15 min.
1X	PBS with 1% T x 100	15 min.

NOTE: Make sure that 3D cultures have settled to the bottom of the microplate well before exchanging solutions.

Labeling

		3D Cell Culture
1X	Penetration buffer	15 min.
1X	Blocking buffer	30 min.
1X	Antibody buffer	30 min.
	Add nuclear stain(optional)	
5X	1X wash buffer	15 min.
1X	Antibody buffer	30 min.
5X	1X wash buffer	15 min.
	Add secondary antibody (optional)	
1X	PBS	15 min.

NOTE: Antibody incubation time will vary from 30 minutes to 12 hours depending on the model.

Clearing

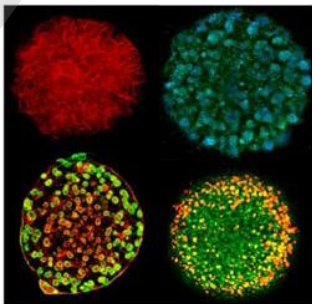
		3D Cell Culture
1X	MeOH	15 min.
1X	Corning 3D Clear Reagent	15 min.

Included with the Starter Kit

- Penetration buffer
- Blocking buffer
- Antibody buffer
- 10X wash buffer
- Corning 3D Clear reagent

Imaging

1. Once cleared with Corning 3D Clear Tissue Clearing Reagent, the 3D cell culture models should be left in a microplate or transferred to a slide in 3D Clear Tissue Clearing Reagent for imaging.
2. 3D cell culture models cleared with Corning 3D Clear Tissue Clearing Reagent can be imaged with a confocal or widefield microscopy. While Corning 3D Clear Tissue Clearing Reagent will allow for a substantial increase in the number of cells characterized with widefield microscopy, optical Z sectioning will not be possible as it is with confocal microscopy.
3. The ideal imaging systems for imaging 3D cell culture models are high content confocal imaging systems such as the Opera Phenix®/Operetta (PerkinElmer), CellInsight™ CX7 LZR/LED (Thermo Fisher), IN Cell analyzer 6000/6500 (GE), or the ImageXpress® (Molecular Devices).
4. For optimum image quality, use glass, flat-bottom microplates (Corning 4580) or Corning spheroid microplates (Corning 4515, 4520) in a high content confocal imaging system with laser excitation.



For more specific information on claims, visit the Certificates page at **www.corning.com/lifesciences**.

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