Corning® 3D Clear Tissue Clearing Reagents and Buffers

Frequently Asked Questions

CORNING

Product Specifications

1. **Do I have to purchase the whole starter kit if I only need the clearing reagent?**

   All of the Corning 3D Clear tissue clearing reagent starter kit components can be purchased separately.

Cat. No.	Description	Qty/Cs
5730	Corning 3D Clear tissue clearing reagent starter kit includes: Corning 3D Clear reagent (30 mL), Corning 3D Clear antibody buffer (30 mL), Corning 3D Clear blocking buffer (30 mL), Corning 3D Clear penetration buffer (30 mL), Corning 3D Clear washing buffer 10X (70 mL)	1
5731	Corning 3D Clear tissue clearing reagent, 10 mL	1
5732	Corning 3D Clear tissue clearing reagent, 30 mL	1
5733	Corning 3D Clear tissue clearing reagent, 100 mL	1
5734	Corning 3D Clear antibody buffer, 30 mL	1
5735	Corning 3D Clear antibody buffer, 100 mL	1
5736	Corning 3D Clear blocking buffer, 30 mL	1
5737	Corning 3D Clear blocking buffer, 100 mL	1
5738	Corning 3D Clear penetration buffer, 30 mL	1
5739	Corning 3D Clear penetration buffer, 100 mL	1
5740	Corning 3D Clear washing buffer 10X, 70 mL	1
5741	Corning 3D Clear washing buffer 10X, 200 mL	1

2. **What are the shipping and storage conditions for the tissue clearing reagent?**

   The Corning 3D Clear tissue clearing reagent is shipped at room temperature and should be stored well-sealed at room temperature in a dry environment. When properly stored, the tissue clearing reagent has a shelf life of 24 months.

3. **What are the compositions of the buffers?**

Buffer Name	Composition	Storage	Shelf Life
Corning 3D Clear penetration buffer	PBS 0.2% Triton™ X-100 0.3 M Glycine 20% DMSO	Room temperature	24 months
Corning 3D Clear washing buffer 10X	10X PBS 0.2% Tween® 20 100 µg/mL Heparin	4°C	24 months. Store up to 6 months once diluted to 1X.
Corning 3D Clear blocking buffer	PBS 0.2% Triton X-100 6% Donkey serum 10% DMSO	4°C	24 months
Corning 3D Clear antibody buffer	PBS 0.2% Tween 20 10 µg/mL Heparin 3% Donkey serum 5% DMSO	4°C	24 months

Detailed instructions to prepare buffers can be found in the Buffer Solutions Recipes for Immunolabeling document (CLS-AN-598).

4. **How many samples can be processed with the Corning® 3D Clear tissue clearing reagent starter kit?**

   The Corning 3D Clear tissue clearing reagent starter kit can be used for processing one to two 96-well microplates or one 384-well microplate at the recommended volume per well of clearing reagent. Otherwise, the number of samples are dependent upon the type of sample being processed because the volumes necessary to adequately cover samples will vary with different sample sizes and dish or well-plate formats. A processing volume at the higher end of the well working volume range will adequately cover many sample cultures but this must be tested for each application.

Applications

5. **How is the Corning 3D Clear tissue clearing technique different than other tissue clearing reagents/methods?**

   A comparison of the numerous established tissue clearing methods reveals that they vary in 6 key areas: processing time, immunolabeling compatibility, fluorescent protein compatibility, tissue integrity, compatibility with well plates (for throughput processing), and reversibility. Protein hyper-hydration (Scale, Clear^{T2}, CUBIC) and hydrogel embedding (PACT/PARS, CLARITY) techniques take hours to days for clearing and are very damaging to tissue integrity (i.e., induce tissue expansion). The complicated processing also makes these particularly unsuitable for throughput imaging/screening applications in well-plate formats. Aqueous refractive index matching (SeeDB/FRUIT) does maintain tissue integrity, but requires hours for the clearing step and is not reversible.

   The Corning 3D Clear tissue clearing technique falls under the solvent-based clearing category, which are the fastest of clearing methods. Many other solvent-based clearing reagents (BABB, i/3DISCO) cause tissue shrinkage, are not compatible with well plates, and are not reversible. The Corning 3D Clear tissue clearing reagent is strong enough to adequately clear samples without significantly changing cell/tissue morphology and has been demonstrated for use with 3D cell culture models, which cannot be said of all tissue clearing techniques. The Corning 3D Clear tissue clearing technique is rapid, reversible, and compatible with microplate imaging, fluorescent protein, and immunofluorescence.

6. **Can the tissue clearing reagent be used to clear spheroids expressing fluorescent proteins?**

   The Corning 3D Clear tissue clearing technique is compatible with immunofluorescence, fluorescent proteins, and other fluorescent labels. For spheroids expressing fluorescent protein, the protocol is simple. Following fixation (optional) and application of nuclear/viability stains (optional), samples are dehydrated with ethanol, and then cleared with 3D Clear tissue clearing reagent.

7. **Is the tissue clearing reagent only for 3D cell cultures, or can it also be used to clear tissues or 2D cell cultures?**

   The Corning 3D Clear tissue clearing reagent can be used to clear spheroids, organoids, 2D cell cultures, or tissue sections that are <500 µm thickness. The current recommended protocol timing and reagent/buffer volumes are based on clearing of 3D cell cultures and may require adjustment for full clearing of tissues and 2D cultures.

8. **Is there a protocol for clearing of spheroids/organoids embedded in a gel (e.g., agarose, collagen, Corning Matrigel® matrix)?**

   Scaffold-based 3D cell cultures can be cleared with the Corning 3D Clear tissue clearing technique, but they must be free of the scaffold before clearing. Some small spheroids or those models that have generated enough of their own extracellular support matrix may maintain enough structure to withstand removal or dissolution of the hydrogel and subsequent clearing. If the 3D culture model requires the scaffold to remain intact, it would require an alternative clearing method.

Protocol

9. **Are there any tips or tricks for clearing large, dense spheroids?**

   The Corning 3D Clear tissue clearing technique is intended for processing spheroids <500 µm thickness. Some spheroids/organoids may require additional permeabilization steps. Alcohol dehydration following fixation permeabilizes samples and can help with solution transfer. Extending the incubation in the Corning 3D Clear tissue clearing reagent by 30% to 50% may also be necessary to achieve full clearing depending on the degree of sample fixation.

10. How long is the clearing protocol for spheroids?

Spheroid clearing is generally a quick process. The recommended protocol specifies a 15-minute incubation in Corning® 3D Clear tissue clearing reagent, but the incubation time may need to be extended by 30% to 50% depending on spheroid density. Duration of the protocol start-to-finish from fixation, staining or immunolabelling, dehydration, through clearing typically takes several hours, depending upon the duration of rate-limiting fixation and immunolabeling steps. There are several stopping points to pause spheroid processing during the protocol.

11. Is dehydration necessary for successful clearing?

There are 2 alcohol dehydration steps. The first dehydration step following fixation to permeabilize tissues can help with solution transfer, especially with large or dense samples. If not combining the clearing with immunolabeling or if antibody penetration is not an issue, this dehydration step can be omitted. However, the second dehydration step of sequential washes of increasing concentrations of ethanol (or methanol), which occurs just before clearing, is critical for tissue clearing.

12. Is there a change in the degree of clearing if I use a larger volume of clearing reagent? Is there a minimal volume I need to use?

In the clearing step, there must be adequate volume to fully cover the sample, which can vary depending upon 3D model size. We recommend 200 µL per well for 96-well microplates and 75 µL per well for 384-well microplates. Increasing the volume beyond this amount will not significantly enhance or accelerate clearing.

13. How does clearing reversal affect spheroids/organoids?

The Corning 3D Clear tissue clearing technique is not destructive to cell or tissue morphology and thus can be fully reversed without changes to the spheroids/organoids. To reverse, samples are washed repeatedly (>10X) with absolute or histological grade ethanol and incubated at room temperature until spheroids become opaque. Samples can then be processed directly for traditional histology. Spheroids/organoids can be rehydrated for paraffin embedding by sequential 15-minute washes of decreasing ethanol concentrations (90% ethanol, 70% ethanol, 50% ethanol, 30% ethanol, 10% ethanol, in PBS) followed by ≥5X PBS washes.

14. Where can I find more information on the 3D Clear tissue clearing technique and applications of tissue clearing?

Additional information can be found on www.corning.com/lifesciences:

- Corning 3D Clear Tissue Clearing Reagent Product Information Sheet (CLS-AC-024)
- 3D Imaging of Optically Cleared Spheroids in Corning Spheroid Microplates Application Note (CLS-AN-509)
- Buffer Solution Recipes for Immunolabeling (CLS-AN-598)
- Corning 3D Clear Tissue Clearing Reagent Guidelines for Use (CLS-AN-600DOC)
- Corning 3D Clear Tissue Clearing Reagent Quick Start Guide (CLS-AN-601DOC)
- Clearing Spheroids with Corning 3D Clear Tissue Clearing Reagent in Corning Spheroid Microplates Guidelines for Use (CLS-AN-602DOC)
- 3D Cell Cultures and Tissue Clearing Webinar
- 3D Cell Culture and the Quest for Effective NASH/NAFLD Solution Webinar

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