Corning® 3D Clear Tissue Clearing Reagent

Guidelines for Use

CORNING

The Corning 3D Clear tissue clearing technique is designed specifically for use with 3D cell cultures, and clears them with minimal changes to morphology and without compromising the sensitivity of detection with almost any fluorophore. With this easy-to-use protocol, 3D cell cultures up to 500 µm in thickness can be cleared within minutes, without using any special instrument or equipment. The clearing workflow is compatible with most fluorophores including fluorescent proteins, which can be detected with typical fluorescent imaging instruments such as wide-field, confocal, and light sheet microscopes, and high-content analyzers. The Corning 3D Clear tissue clearing technique is strong enough to adequately clear tissue for 3D fluorescent imaging, but not so harsh as to change the overall tissue morphology. Minimum morphological changes such as shrinkage or contraction have been observed. For precious samples, the clearing can be reversed, and tissue can be processed for histology studies such as H&E staining.

Table 1. Contents and storage of Corning 3D Clear Reagent Starter Kit

Corning 3D Clear Reagent Starter Kit (Cat. No. 5730)

Includes:	Size (mL)
Corning 3D Clear Reagent	30
Corning 3D Clear Antibody Buffer	30
Corning 3D Clear Blocking Buffer	30
Corning 3D Clear Antibody Penetration Buffer	30
Corning 3D Clear Washing Buffer, 10X	70

Upon receipt of the starter kit:

- 3D Clear Reagent should be stored well-sealed at room temperature in a dry environment. Do not freeze. When stored as directed, it is stable for 24 months from the date of receipt.
- 3D Clear buffers should be stored in a refrigerator (2°C to 8°C) upon receipt and are stable for 12 months from the date of receipt.

For 3D cell culture models up to 500 µm in thickness, we recommend using Corning 3D Clear reagent. For first-time users, we recommend the Corning 3D Clear Starter Kit (Cat. No. 5730) for 3D cell culture models, which contain all reagents required for the workflows described here.

Table 2. Contents and storage of stand-alone Corning 3D Clear reagents

Description	Cat. No.	Size (mL)	Description	Storage
Corning 3D Clear Reagent	5731 5732 5733	10 30 100	Clearing and imaging reagent with a refractive index of 1.48	Room temperature in a dry environment.* **Do not freeze.**
Corning 3D Clear Antibody Buffer	5734 5735	30 100	PBS with 0.2% TWEEN® 20, heparin, 3% donkey serum, and 5% DMSO	2°C to 8°C**
Corning 3D Clear Blocking Buffer	5736 5737	30 100	PBS with 0.2% Triton™ X-100, 6% donkey serum, and 10% DMSO.	2°C to 8°C**
Corning 3D Clear Penetration Buffer	5738 5739	30 100	PBS with 0.2% Triton X-100, 0.3 M glycine, and 20% DMSO	
Corning 3D Clear Washing Buffer, 10X	5740 5741	70 200	10X PBS with 2% TWEEN 20 and 100 µg/mL heparin	

*When stored as directed, the products are stable for 24 months from the date of receipt.
**When stored as directed, the products are stable for 12 months from the date of receipt.

Materials Required (not supplied)

- 3D cell models (e.g., organoids, spheroids)
- Slides, coverslips, containers
- Primary and secondary antibodies
- PBS (phosphate buffered saline), pH 7.4 (without calcium, magnesium, or phenol red)
- Ethanol (for samples containing fluorescent proteins)
- Methanol (for samples without fluorescent proteins)
- DMSO, Anhydrous
- 4% formaldehyde, methanol-free
- PBS with 0.05% sodium azide (**CAUTION:** Sodium azide is extremely toxic!)

Before You Begin

Procedural Guidelines

- For 3D cell culture models up to 500 µm thickness, use Corning 3D Clear reagent in the clearing step.
- Corning 3D Clear tissue clearing reagent may be used with a fluorescent imaging system.
- Best results are obtained with 3D cell models that have been fixed for 30 minutes with 10% neutral buffered formalin at room temperature. Remove excess formalin from wells and replace with PBS. If long-term (>1 week) storage is required prior to labeling and clearing, transfer 3D cell models to PBS with 0.05% sodium azide as a preservative. Otherwise, leave 3D cell models in PBS and proceed with further processing.
- Except where otherwise stated, perform all steps in the procedure at room temperature (20°C) with gentle agitation. If autofluorescence is a significant problem with your models, conduct all steps at 4°C and use 100% dry ethanol instead of methanol.
- For 3D cell culture models, tissue dehydration is not necessarily required, but does enhance tissue clearing, especially for dense 3D cell culture models (e.g., neuronal models).
- For all steps involving ethanol, use 100% water-free ethanol. Reagent alcohol is a suitable choice (Fisher Scientific HC-600-1GAL), containing 90% ethanol, 5% isopropanol, and 5% methanol.
- You can perform all steps within polystyrene multiwell microplates. We recommend use of optical bottom multiwell microplates for imaging; however Corning spheroid microplates featuring Ultra-Low Attachment (ULA) coating are also compatible for processing and imaging.

1.0 Prepare the Reagents

1.1 Corning 3D Clear 10X Washing Buffer is provided at 10X concentration. Before use, dilute the Corning 3D Clear 10X Washing Buffer to 1X with deionized water.

1.2 For samples containing fluorescent proteins, prepare 30% and 50% ethanol solutions by diluting a higher concentration ethanol solution in PBS, pH 7.4. Prepare 70% and 90% ethanol solutions by diluting a higher concentration ethanol solution in deionized water. **NOTE:** For best results, ensure that the 100% ethanol used in the last step of dehydration is completely dehydrated.

1.3 For samples without fluorescent proteins, prepare 50% methanol solution by diluting a higher concentration methanol solution in equal parts PBS and DI to make a 50% methanol, 25% PBS and 25% DI solution, pH 7.4. Prepare 70% and 90% methanol solution by diluting a higher concentration methanol solution in deionized H_2O. **NOTE:** For best results, ensure that the 100% methanol used in the last step of dehydration is completely dehydrated.

1.4 For samples with extensive pigmentation (liver, kidney), prepare ice-cold 5% H_2O_2 in 20% DMSO/methanol (1 part 30% H_2O_2, 1 part 100% DMSO, 4 parts 100% methanol). **NOTE:** Bleaching with this solution is not compatible with fluorescent protein staining.

2.0 Protocol for Fluorescent Protein-labeled Tissue

The following protocol describes a general procedure for clearing a variety of 3D cell culture models (e.g., organoids, microtissues, spheroids). The procedure is effective at clearing 3D cell models fixed with a variety of fixatives, and 3D cell models that have been stored in formalin for years. Refer to Table 3 for the suggested incubation times and volumes.

Table 3. Incubation times and reagent volumes required for clearing fluorescent protein or fixable fluorophore-labeled 3D cell models

	Ethanol Dehydration	Volume of Ethanol for Each Step	Incubation Time in 3D Clear Tissue Clearing Reagent	Volume of 3D Clear Tissue Clearing Reagent*
3D Cell Culture Models	15 minutes	75 µL (384-well)	15 minutes	75 µL (384-well)
	15 minutes	200 µL (96-well)	15 minutes	200 µL (96-well)

*For especially dense or large models, incubation time may need to be extended by 30% to 50%, depending on degree of fixation.

Except where otherwise stated, perform all steps in the procedure at room temperature with gentle agitation.

Obtain 3D cell models of interest. See Procedural Guidelines section for guidelines on fixation.

2.1 Wash 3D cell models twice in PBS, pH 7.4 (without calcium, magnesium, or phenol red) for at least 15 minutes. **STOPPING POINT.** (Optional) You can store the 3D cell models at 4°C in the dark for up to 3 days without detrimental effects.

2.2 Dehydrate the 3D cell models with increasing concentrations of ethanol at 4°C. See Table 3 for required volumes and incubation times. Using an excess volume in the dehydration steps ensures proper clearing.

NOTE: For 3D cell culture models, tissue dehydration is not required, but it can enhance tissue clearing speed for dense or especially large 3D cell culture models (e.g., neuronal models).

2.2.1 Treat 3D cell models with 30% ethanol in PBS with gentle shaking.

2.2.2 Treat 3D cell models with 50% ethanol in PBS with gentle shaking.

2.2.3 Treat 3D cell models with 70% ethanol in deionized water with gentle shaking.

2.2.4 Treat 3D cell models with 90% ethanol in deionized water with gentle shaking.

2.2.5 Treat 3D cell models with 100% dry ethanol with gentle shaking.

2.3 Remove as much ethanol as possible from the sample via a pipettor.

2.4 Add Corning 3D Clear reagent, incubate for 15 minutes, then proceed to imaging.

NOTE: Required reagent volume and clearing time vary with 3D model size (see Table 3). Clearing can be accelerated at room temperature at the cost of decreased endogenous fluorescence from fluorescent protein.

NOTE: For 3D cell culture models, use only Corning 3D Clear reagent for tissue clearing.

NOTE: DAPI gives much better results with cleared samples and is preferable to Hoechst 33342.

STOPPING POINT. You can seal and store the cleared samples at 4°C in the dark indefinitely without detrimental effects. Depending on the sample type and the fluorophore, mounted samples can be imaged weeks to months after clearing. Depending on the length of storage, you might need to restain with a nuclear stain.

2.5 Image the cleared samples using any fluorescent imaging analyzer such as widefield microscope, confocal, light sheet or single/multi-photon microscope, or high content analyzer. You can image the samples in any appropriate container, such as mounted slides, 96-well microplates, light sheet microscope chambers, etc.

3.0 Protocol for Immunolabeling 3D Cells Models

The following protocol describes a general procedure for immunolabeling and clearing a variety of 3D cell culture models (e.g., organoids, microtissues, spheroids). Refer to Table 4 for the suggested incubation times and Table 5 for the required reagent volumes to immunolabel and clear your model of interest.

Table 4. Suggested incubation times for immunolabeling and clearing 3D cell culture models

	Permeabilization and Dehydration Steps	Penetration Buffer	Blocking*	Antibody Incubation*	Washing Steps	Incubation in Corning 3D Clear Reagent*
3D Cell Culture Models	15 minutes	30 minutes	30 minutes	1 hour	15 minutes	15 minutes

*For especially dense or large models, depending on degree of fixation, incubation time may need to be extended by 30% to 50%.

Table 5. Reagent volumes required for immunolabeling and clearing 3D cell culture models

	Permeabilization and Dehydration	Penetration/ Permeabilization/Washing	Blocking/Antibody Incubation	Clearing
96-well microplate	200 µL	200 µL	200 µL	200 µL
384-well microplate	75 µL	75 µL	75 µL	75 µL

Except where otherwise stated, perform all steps in the procedure at room temperature (20°C) with gentle agitation.

3.1 Obtain 3D cell models of interest and fix them, if needed. See Procedural Guidelines section for guidelines on fixation.

3.2 Wash 3D cell models twice in PBS, pH 7.4 (without calcium, magnesium, or phenol red) for at least 15 minutes each.

NOTE: For 3D cell culture models that are particularly difficult to immunolabel due to the spheroids being highly dense in nature or the presence of significant ECM, a permeabilization buffer (e.g., HSK-PMB-3D from Visikol) can be used. Incubate at room temperature for 15 to 30 minutes with gentle shaking, and wash several times with PBS to remove as much buffer as possible before proceeding with next steps. ***Please note that Visikol Permeabilization Buffer should not normally be needed for most 3D cell culture models.***

NOTE: Visikol Permeabilization Buffer cannot be used if immunolabeling 3D cell models contain fluorescent protein.

3.3 Permeabilize 3D cell models by washing them through a gradient of methanol (samples without fluorescent protein) at room temperature, or ethanol (samples with fluorescent protein) at 4°C with gentle agitation. See Tables 4 and 5 for required volumes and incubation times.

- **a. Samples without fluorescent protein:** Wash 3D cell models twice in PBS, once in 50% methanol with 25% PBS and 25% DI, once in 80% methanol in deionized water, and finally once in 100% dry methanol.
- **b. Samples with fluorescent protein:** Wash 3D cell models twice in PBS, 50% methanol in PBS, once in 80% ethanol in deionized water, and finally once in 100% dry ethanol (conduct at 4°C).

STOPPING POINT. (Optional) You can store the tissues in methanol (samples without fluorescent protein) or ethanol (samples with fluorescent protein) at 4°C for up to 2 weeks without detrimental effects.

3.4 (Optional) Bleach tissues containing substantial quantities of blood or pigment (such as non-perfused heart, lung, kidney, or liver tissue) by submerging them in ice-cold 5% H_2O_2 in 20% DMSO/methanol (1 part 30% H_2O_2, 1 part 100% DMSO, 4 parts 100% methanol) and incubating at 4°C overnight. This step significantly reduces background fluorescence caused by hemoglobin.

3.5 Wash samples before proceeding with further staining:

- **a. Samples without fluorescent protein:** Wash the 3D cell models once in 20% DMSO/methanol, once in 80% methanol in deionized water, once in 50% methanol with 25% PBS and 25% DI, once in 100% PBS, and finally in PBS with 0.2% Triton™ X-100.
- **b. Samples with fluorescent protein:** Wash the 3D cell models in 20% DMSO/ethanol, in 80% ethanol in deionized water, in 50% ethanol in PBS, in 100% PBS, and finally in PBS with 0.2% Triton X-100 (conduct at 4°C).

STOPPING POINT. (Optional) Before storing, transfer the samples to 100% PBS (without Triton X-100). You can store the 3D cell models at 4°C in the dark for up to 3 days without detrimental effects.

3.6 Incubate the samples in Corning 3D Clear Penetration Buffer with gentle shaking.

3.7 Block the samples in Corning 3D Clear Blocking Buffer with gentle shaking at 37°C. For samples containing fluorescent protein, incubate at 4°C.

STOPPING POINT. (Optional) Before storing, transfer the samples to 100% PBS. You can store the 3D cell models at 4°C for up to 1 month without detrimental effects.

3.8 Transfer the samples to primary antibody dilutions prepared in Corning 3D Clear Antibody Buffer, and incubate at 37°C with gentle shaking. For samples containing fluorescent protein, incubate at 4°C.

NOTE: For most broadly expressing epitopes, a dilution of 1:50 to 1:500 is typically required, but antibody concentration should be optimized for 3D cell models according to the Guidelines for Validating Antibodies and Optimizing Antibody Concentration (Appendix A).

3.9 Wash the samples 5 times in Corning 3D Clear Washing Buffer (diluted to 1X with DI H_2O; see Step 1.1) with gentle shaking.

STOPPING POINT. (Optional) Before storing, transfer the samples to 100% PBS. You can store the 3D cell models at 4°C for up to 2 weeks without detrimental effects.

3.10 If using secondary antibody detection, incubate the samples in secondary antibody dilutions (1:50 to 1:500, depending on the dilution of the primary antibody) in Corning 3D Clear Antibody Buffer at 37°C with gentle shaking.

STOPPING POINT. (Optional) Before storing, transfer the samples to 100% PBS. You can store the 3D cell models at 4°C for up to 2 weeks without detrimental effects.

3.11 (Optional) Add nuclear stain (e.g., DAPI) to a dilution of 1:1000 to 1:5000 (depending on the stain). You can perform this step concurrently with antibody labeling steps, or separately in Corning 3D Clear Washing Buffer. DAPI gives better results and should be used as a nuclear stain instead of Hoechst 33342.

3.12 Wash the samples 5 times in Corning 3D Clear Wash Buffer with gentle shaking. You can keep the samples in Corning 3D Clear Wash Buffer indefinitely before proceeding with the subsequent steps.

NOTE: Samples which have **not** been stained with antibodies normally require only 3 washes. In 384-well microplates, due to the difficulty in removal of all liquid within the wells, an increased number of washes should be performed (e.g., 7 to 10 washes). If excess background staining still occurs, increase the number of washes.

STOPPING POINT. (Optional) You can store the 3D cell models at 4°C in the dark for up to 3 days without detrimental effects.

NOTE: For 3D cell culture models, tissue dehydration is not required, but it can enhance tissue clearing speed for dense 3D cell culture models (e.g., neuronal models).

STOPPING POINT. (Optional) You can store the tissues at 4°C in the dark for up to 3 days without detrimental effects.

3.13 Dehydrate the 3D cell models with increasing concentrations of methanol (samples without fluorescent protein) or ethanol (samples with fluorescent protein) at 4°C with gentle shaking. See Tables 4 and 5 for required volumes and incubation times. Using an excess volume in the dehydration steps ensures proper clearing.

- **a. Samples without fluorescent protein:** Treat 3D cell models with 50% methanol with 25% PBS and 25% DI, then with 80% methanol in deionized water, and finally in 100% methanol with gentle shaking.
- **b. Samples with fluorescent protein:** Treat 3D cell models with 50% ethanol in PBS, then with 80% ethanol in deionized water, and finally in 100% ethanol with gentle shaking at 4°C.

STOPPING POINT. (Optional) You can store the 3D cell models at 4°C for up to 3 days without detrimental effects.

3.14 Remove as much methanol/ethanol as possible from sample.

3.15 Add Corning 3D Clear, incubate for 15 minutes (may require longer incubation time for thicker 3D cell models), then proceed to imaging (conduct at 4°C for samples with fluorescent protein).

NOTE: Required reagent volume and clearing time vary with 3D cell model sample size (see Table 3). However, tissue clearing can be accelerated substantially at 37°C with gentle shaking without damage to tissue, at the compromise of increased autofluorescence. Do not use higher temperature incubation with samples containing fluorescent protein.

STOPPING POINT. You can seal and store the cleared samples at 4°C in the dark indefinitely without detrimental effects. Depending on the sample type and the fluorophore, mounted samples can be imaged weeks to months after mounting.

3.16 Image the cleared samples using confocal, light sheet, or single or multi-photon microscopy.

APPENDIX A: Troubleshooting

Observation	Possible Cause	Recommended Action
Cannot image past a few cell layers. Labeling appears very intense on the outside of the model but cannot see any staining deep into the model.	3D cell culture model is overfixed or underfixed either causing a lack of antibody penetration or not preserving epitopes	If DAPI labeling is present throughout, overfixation is likely the problem. If not, a lack of fixation is likely the problem. Try several different fixation times and relabel a few 3D cell culture models and look for labeling depth.
	Antibody concentration is too high: ring of intense staining near the surface, drops off significantly after that.	Reduce antibody concentration. If the signal is too weak, use a lower antibody concentration for half of the time, then re-incubate with antibodies at a higher concentration.
	Antibody concentration too low: signal drops off in the middle of the tissue.	Increase antibody concentration.
	Optical attenuation due to absorption of photons by the upper tissue layers "shadows" the tissue below, even with perfect staining.	- Increase laser power and gain with increasing depth. Some microscopes can automate laser power and gain corrections. **CAUTION:** Higher laser power increases the rate of photobleaching. - Ensure the samples does not contain air bubbles. - Compare intensity loss to nuclear stain intensity. Because nuclear stain diffuses very fast into 3D cell models, you can use this signal to correct for signal loss in image processing.
There is an intense band of labeled tissue at the surface, then a significant drop-off afterwards.	Antibody concentration is too high.	Reduce antibody concentration by increasing the dilution factor.
Tissue did not clear.	Plastic incompatibility.	Corning 3D Clear tissue clearing reagent is compatible with polystyrene microplates. Be sure to use polystyrene microplates for your application. Most optical bottom microplates are made from polystyrene.
	Incomplete dehydration/clearing	- Ensure that you are using pure, dehydrated ethanol or methanol for drying. Impure methanol or ethanol that contains water will not remove all of the water from the tissue, resulting in cloudiness. - Ensure that the sample vessel is sealed properly. Corning 3D Clear tissue clearing reagent is hygroscopic and will absorb water from the air. - Due to the difficulty in removal of all liquid from well plates, there may have been some alcohol left behind before adding Corning 3D Clear tissue clearing reagent. Remove the Corning 3D Clear tissue clearing reagent from the well, and add a fresh volume.
Fluorescent protein is quenched.	Sample containing fluorescent protein is dehydrated using methanol.	To visualize fluorescent proteins, samples must be dehydrated using ethanol at 4°C instead of methanol.
	Sample is bleached.	- Keep cleared samples in the dark and cover them with aluminum foil, because fluorescent proteins photobleach rapidly when exposed to ambient light. - Do not treat fluorescent protein-labeled samples with H_2O_2. This step oxidizes fluorescent proteins, resulting in loss of signal. - Do not treat fluorescent protein-labeled samples with permeabilization buffers.
	Background fluorescence too high.	Conduct all steps in the protocol to 4°C and increase their duration by 50%.
Antibody did not label the tissue.	Antibody is not compatible with 3D immunolabeling.	- Validate the specificity of your antibody on small tissue sections before proceeding to larger 3D cell models. Contact Corning Scientific Support if you have any questions about your specific antibody. - Only use antibodies that have been validated for use in IHC. If IHC validated antibody is not available, then IF/ ICC validated antibody might also work.
Center of 3D models look dark.	Antibody concentration is too low.	Increase the antibody concentration. Explore a range of antibody concentrations on a small section of the tissue before scaling to large 3D cell models.
	Optical attenuation.	- Optical attenuation leads to diminished signal at increasing depths depending on several factors, such as concentration of label bound in upper layers of the tissue, level of autofluorescence, type of objective, and laser power. - Modify laser power and gain according to tissue depth to account for optical attenuation. This can be automated in systems such as the Leica SP5 and SP8. - Histogram matching during image processing can account for optical attenuation at the cost of increased noise at greater depths.

APPENDIX B: Guidelines for Validating Antibodies and Optimizing Antibody Concentration

If you are using an antibody for the first time, we recommend that you validate the antibody and optimize its concentration.

- Fix the 3D cell models with 10% neutral buffered formalin at room temperature for 30 minutes. Do not over-fix the 3D cell models.
- Label 3D cell models using various concentrations of the primary antibody, ranging from 1:50 to 1:500 (e.g., 1:50, 1:100, 1:200, 1:300, 1:500), diluted in Corning 3D Clear Antibody Buffer.
- Typically, 1:100 dilution of the secondary antibody works well. However, you might have to optimize the secondary antibody concentration if you observe low signal or high background.
- To evaluate the evenness of staining, image the 3D cell models using a confocal microscope. Obtain a z-stack image spanning the entire thickness of the tissue section using two color channels: the channel corresponding to the fluorescent conjugate for antibody staining, and the channel used for nuclear stain. Because nuclear stains penetrate 3D cell models rapidly and homogeneously, the nuclear stain channel serves as a control for optical attenuation.
- Optical attenuation will naturally cause deeper layers of 3D cell models to appear dimmer than the outer layers; this can be corrected by normalizing the intensity of each slice in the Z stack.
- Examine the z-stacks in ImageJ program (or other image processing software). Observe the XZ and YZ planes by viewing “Orthogonal Views” and examine the evenness of staining.
- If the staining is even, you should see relatively consistent intensity (with respect to nuclear stain) across the tissue (Figure 1). Some dimming in the inner layers is expected, but signal should be visible across tissue.
- If the concentration of the immunolabel is too high, you will see a bright ring of staining at the surface layers, with uneven staining at a lower intensity deeper into the tissue.
- If the concentration of the immunolabel is too low, you will see slight staining at the surface layer, a dark interior, and uneven spots of stain.

APPENDIX C: Reverse Tissue Clearing

The Corning 3D Clear clearing process is non-destructive and reversible, allowing traditional 2D histology to be conducted after 3D imaging. Because of the reversible nature of this approach, the Corning 3D Clear clearing method can be integrated into many bio-imaging processes without disrupting traditional workflows or histological processing.

- Wash 3D cell models repeatedly (at least 10X) with absolute or histological grade ethanol. Leave tissue at room temperature until opacity has been restored.
- After reversal, samples can be processed directly for paraffin-embedding histological preparations.
- 3D cell models can be rehydrated by washing through a gradient of ethanol and PBS (90% ethanol, 70% ethanol, 50% ethanol, 30% ethanol, 10% ethanol, in PBS), with 3X washes each step, incubating for 15 minutes for each wash. Finally, wash the samples with PBS at least 5X.

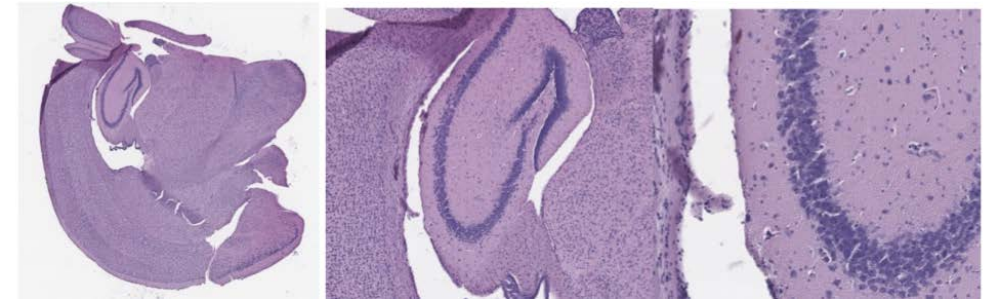

Figure 1A. Untreated mouse brain tissue section was formalin-fixed and paraffin-embedded, then stained with H&E, depicting the hippocampus.

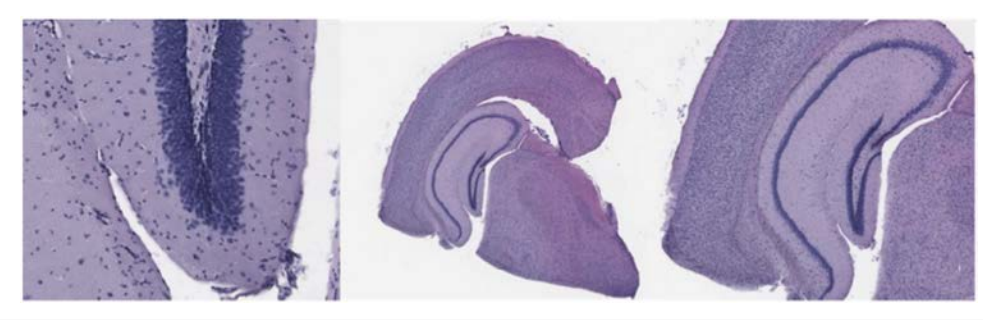

Figure 1B. The mouse brain tissue was cleared using the Corning 3D Clear tissue clearing technique. Cleared tissue was then reversed, embedded in paraffin, sectioned, and stained with H&E, depicting hippocampus. The Corning 3D Clear workflow does not appreciably affect tissue histology.

Figure 1C. Mouse brain tissue was cleared using the Corning 3D Clear tissue clearing technique. Cleared tissue was then reversed, embedded in paraffin, sectioned, and immunostained for GFP, labeling astrocytes. The Corning 3D Clear tissue clearing workflow does not affect antigenicity of tissues.

Ordering Information

Cat. No.	Description	Size (mL)
5730	Corning 3D Clear Tissue Clearing Starter Kit Includes 10 mL sizes of each of the following: Corning 3D Clear Tissue Clearing Reagent Corning 3D Clear Antibody Buffer Corning 3D Clear Blocking Buffer Corning 3D Clear Penetration Buffer Corning 3D Clear Washing Buffer 10X	1 kit
5731	Corning 3D Clear Tissue Clearing Reagent	10
5732	Corning 3D Clear Tissue Clearing Reagent	30
5733	Corning 3D Clear Tissue Clearing Reagent	100
5734	Corning 3D Clear Antibody Buffer	30
5735	Corning 3D Clear Antibody Buffer	100
5736	Corning 3D Clear Blocking Buffer	30
5737	Corning 3D Clear Blocking Buffer	100
5738	Corning 3D Clear Penetration Buffer	30
5739	Corning 3D Clear Penetration Buffer	100
5740	Corning 3D Clear Washing Buffer, 10X	70
5741	Corning 3D Clear Washing Buffer, 10X	200

Customer and Technical Support

Visit **www.corning.com/3Dclear** for the latest in services and support, including:

- Contact telephone numbers
- Product Support, including:
  - Product Frequently Asked Questions
- Order and Web Support
- Product documentation, including:
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets

The information in this guide is subject to change without notice.

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

Warranty/Disclaimer: Unless otherwise specified, all products are for research use only. Not intended for use in diagnostic or therapeutic procedures. Not for use in humans. Corning Life Sciences makes no claims regarding the performance of these products for clinical or diagnostic applications.

For additional product or technical information, visit **www.corning.com/lifesciences** or call 800.492.1110. Outside the United States, call +1.978.442.2200 or contact your local Corning sales office.

CORNING

Corning Incorporated
Life Sciences
836 North St.
Building 300, Suite 3401
Tewksbury, MA 01876
t 800.492.1110
t 978.442.2200
f 978.442.2476
www.corning.com/lifesciences

ASIA/PACIFIC

Australia/New Zealand
t 61 427286832

Chinese Mainland
t 86 21 3338 4338
f 86 21 3338 4300

India
t 91 124 4604000
f 91 124 4604099

Japan
t 81 3-3586 1996
f 81 3-3586 1291

Korea
t 82 2-796-9500
f 82 2-796-9300

Singapore
t 65 6572-9740
f 65 6735-2913

Taiwan
t 886 2-2716-0338
f 886 2-2516-7500

EUROPE
CSEurope@corning.com

France
t 0800 916 882
f 0800 918 636

Germany
t 0800 101 1153
f 0800 101 2427

The Netherlands
t 020 655 79 28
f 020 659 76 73

United Kingdom
t 0800 376 8660
f 0800 279 1117

All Other European Countries
t +31 (0) 206 59 60 51
f +31 (0) 206 59 76 73

LATIN AMERICA
grupoLA@corning.com

Brazil
t 55 (11) 3089-7400

Mexico
t (52-81) 8158-8400