

ADAPT-3D™ Organoid Clearing Kit Product Insert



Plate-based optical clearing and whole-mount immunolabeling of 100–500 µm organoids

Catalog Number A640

Product Information:

ADAPT-3D (Accelerated Deep Adaptable Processing of Tissue for 3-Dimensional Imaging)

The acronym ADAPT-3D reflects the adaptable and versatile nature of this protocol across a wide range of model systems. This version is tailored for organoids. The protocol is a guide and should be optimized for organoid size and density. Recommended incubation times for fixation, decolorization, delipidation, and refractive index matching (RIM) are included at the end of the protocol.

NOTE: Incubation times vary with organoid size; reference the tables for approximate incubation times and suggested volumes.



WARNING: Read the Safety Data Sheet for every component before use. Wear appropriate protective eyewear, clothing, and gloves. **Partial Delipidation Buffer B (B676) is corrosive to plastic, polystyrene, PEG, and HDPE.** Use glass vessels only for the delipidation step. Immunostaining Buffer (B673) and Wash Buffer (B674) contain 0.09% sodium azide.

Scaling Down: Standard Tissue vs. Custom Organoid Workflow

Standard Tissue Protocol



Target Scale:
Whole Organs



Fixation:
Transcardiac Perfusion



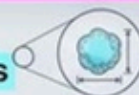
Processing Vessel:
Scintillation Vials

Delipidation Step:
Mandatory

Custom Organoid Workflow



Target Scale:
100–500 µm Organoids



Fixation:
**Immersion in 4% PFA
pH 9.0 (10% Sucrose)**



Processing Vessel:
96-Well U-Bottom Plates

Delipidation Step: **Optional**
(only for >500 µm or lipid-dense samples)

KEY INSIGHT: For most organoids (100–500 µm), **skipping delipidation entirely ensures maximum speed without sacrificing final optical clarity.**

Figure 1: Scaling down from the standard tissue protocol to the organoid workflow. The four changes that matter: target scale, immersion fixation, plate-based processing, and optional rather than mandatory delipidation.

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Kit Components Include:

Product	Cat. No.	Size	Notes	Storage
Decolorization Buffer (ADAPT:DC)	B671	15 mL		• Store at room temperature in a dry environment. • Do not freeze. • When stored as directed, the products are stable for 6 months
Partial Delipidation Buffer (Component A)	B672	5 mL		
Partial Delipidation Buffer (Component B)	B676	5 mL		
Immunostaining Buffer 1X	B673	15 mL	0.1 mM Glycine, 0.167% Tween-20, 0.33% Triton X-100, 1% Donkey Serum, 1% Alpaca Serum, 1% BSA, 0.05% Hydrogen peroxide, 5% v/v DMSO + 0.09% Azide	• Store at 2–8°C. • Follow sterile techniques while using. • When stored as directed, the products are stable for 6 months from the date of receipt.
ADAPT:RI (Refractive Index) Matching (Clearing Solution)	B675	15 mL		• Store at room temperature. • Do not freeze. • When stored as directed, the products are stable for 6 months
Wash Buffer (5X)	B674	20 mL	PBS + 1.5M Glycine + 5U/mL heparin; 0.09% Azide at 1X	• Store at room temperature. • Do not freeze.

Note: ADAPT-3D is stored at multiple temperatures. Store according to product labeling. When stored as directed, the products are stable for 6 months from the date of receipt.

Required Materials Not Included:

1. Organoids cultured in 96-well U-bottom plates; positive and negative controls as required (Use Positive and Negative controls as needed)
2. **Fixative Solution:** 4% paraformaldehyde in PBS, pH 9.0. Add 10% (w/v) sucrose to maximally preserve endogenous fluorescent reporters.
3. PBS (P364), pH 7.4 — to dilute the 5X Wash Buffer and to prepare the 50% RI equilibration solution
4. Primary and secondary antibodies, IHC/IF-validated and confirmed for whole-mount use
5. Glass scintillation vials — only if partial delipidation is performed
6. Glass-bottom or optically clear-bottom 96-well plate and an optically clear seal, for in-plate imaging
7. Wide-bore or pre-cut pipette tips, multichannel pipette, orbital shaker, humidified incubation box

Note: To search through the Leinco Technologies primary antibody collection, use our Antibody Search tool at www.leinco.com.

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Prior to Beginning this protocol:

1. Wash Buffer 2 is supplied as a 5X concentrate (B674). Dilute to 1X in PBS, pH 7.4, before use: 17.3 mL of 5X concentrate plus 69.1 mL of PBS yields the 86.4 mL of 1X buffer, consumed by a full 96-well plate across all six wash fills. If partial delipidation is performed, the extra step-6 wash raises the requirement to 115.2 mL of 1X (23.0 mL of the 5X concentrate), which the 25 mL fill still covers. Prepare the 0.1X dilution in Type 1 ultrapure water separately, and only if partial delipidation is performed.
2. Mix ADAPT:RI (B675) 1:1 with PBS, pH 7.4. A full plate requires 9.6 mL of the mixture — 4.8 mL of B675 plus 4.8 mL of PBS. ADAPT:RI is viscous; equilibrate it to room temperature and pipette slowly to avoid volume error. Cut the end of a pipette tip for easier pipetting. Prepare solution fresh daily.
3. For organoids with endogenous fluorescent reporters, fix in 4% PFA at pH 9.0 with 10% sucrose to preserve, and with 10% (w/v) sucrose to maximally preserve, reporter intensity.

Recommended Workflows

- **Time Savings:** Organoids reach transparency in minutes to a few hours.
- **Organoid size and format:** Most organoids are 100–500 µm in diameter and clear quickly and completely. Organoids are typically grown and processed in 96-well U-bottom plates; fixation, washes, decolorization, staining, and refractive index matching can all be carried out in the plate.
- **Delipidation for deep labeling:** For best immunolabeling, especially for larger organoids (~500 µm in diameter), the partial delipidation (PDL) step is recommended. Because the delipidation buffer is corrosive to plastic, transfer the organoids out of the 96-well plate into a glass vial for this step (see steps 5–6). Smaller, less lipid-dense organoids can often be labeled adequately without delipidation. *Tip: Cut the end of a micropipette tip and use this to core matrigel containing organoids for careful transfer.*
- **Transparency:** Light-interfering molecules (residual pigments and autofluorescent metabolites such as NAD(P)H and flavins) and lipids limit transparency and raise background. Decolorization and delipidation address these; complete transparency is reached only in 100% RI matching solution.
- **Multiple imaging modalities:** Tested with confocal, widefield, and light-sheet microscopy.
- **Mounting:** Cleared organoids can be imaged in any appropriate container, such as the 96-well plate, a glass-bottom dish, mounted slides, or a light-sheet chamber. Keep the organoids in **100%** RI matching solution during imaging.

Procedural guidelines

- Organoids (typically 100–500 µm) need no sectioning; process them intact.
- Perform all steps at room temperature with gentle agitation, except fixation, which is performed at 4 °C.
- Incubation times scale with organoid size and density; larger or denser organoids may need longer decolorization and delipidation.
- Decolorization and delipidation give partial transparency. Complete transparency requires the final 100% ADAPT:RI matching step.
- **Use glass with ADAPT-3D delipidation buffer (B672 and B676). Do not use polystyrene, PEG or HDPE.**
- The workflow is aqueous throughout. Do not dehydrate in methanol, ethanol, or any organic solvent at any point.
- Organoids are immersion-fixed in the plate; perfusion does not apply.

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Plate Setup and Fluid Handling

Plate layout

Reserve rows A and H and columns 1 and 12 as PBS-filled moats rather than sample wells. This leaves 60 usable interior wells per plate for samples and controls and materially reduces evaporation during the long room-temperature incubations. Place a secondary-only column, an unstained column, and positive and negative control wells on the same plate as the experimental samples so that every condition is processed identically and imaged on the same instrument.

Antibody titration

The 50 μL /well immunostaining volume makes the plate a practical titration platform: run a dilution series of the primary antibody across a row while running biological replicates down the columns, and determine the working concentration in a single experiment. Titrate under the exact conditions of the final assay — penetration and background in a whole-mount organoid differ from those on a thin section.

Safe aspiration

Organoids are fragile and easily aspirated. Plate-based processing works only because of disciplined fluid management.

Fluid Dynamics: The Safe Aspiration Technique

Organoids are fragile and easily aspirated. Plate-based processing works because of precise fluid management.

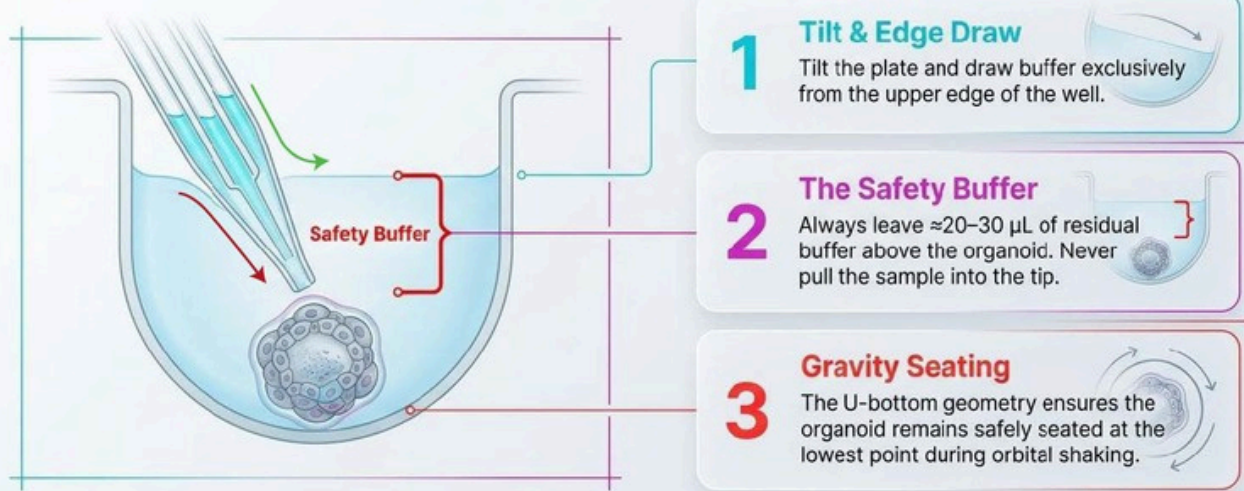


Figure 2: Tilt the plate and draw buffer exclusively from the upper edge of the well. Always leave $\approx 20\text{--}30\ \mu\text{L}$ of residual buffer above the organoid so the sample is never pulled into the tip. The U-bottom geometry keeps the organoid seated at the lowest point during gentle orbital shaking.

- **Tilt and edge-draw.** Never aspirate from the bottom of the well. A multichannel pipette changes a full row of eight wells in one action.
- **Leave the safety buffer.** 20–30 μL above the organoid, every exchange, without exception.
- **Pre-wet and widen tips.** Use wide-bore or pre-cut, pre-wetted tips for any transfer. Chilling the plate on ice for 15–30 min liquefies residual Matrigel and releases organoids with less shearing.
- **Control evaporation.** Keep the lid on, fill the perimeter moat, and incubate in a humidified box during decolorization, delipidation, and overnight antibody steps.

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Standard Organoid Protocol

This version is adapted for Matrigel-embedded organoids. Organoids are immersion-fixed (no perfusion), processed at room temperature except where noted, and require no decalcification. The workflow is aqueous throughout; no acetone or other organic dehydration solvents are used.

Workflow Overview

End-to-end processing without ever moving the sample: fix, wash, decolorize, stain, and clear directly in the 96-well U-bottom plate. Only the optional partial delipidation leaves the plate, and only because Buffer B corrodes plastic.

Step	Reagent	μL / Well	Time & Temperature
1. Fixation	4% PFA, pH 9.0 (+10% sucrose) †	150	≤ 1 h @ 4 °C
2. Wash	1X Wash Buffer (B674)	150	2 × 30 min, RT
3. Decolorization	ADAPT:DC (B671)	150	Day or overnight, RT
4. Wash	1X Wash Buffer (B674)	150	2 × 30 min, RT
5. Delipidation *	PDL A + B, 1:1 — glass vessel	pooled	Overnight, RT
6. Wash *	0.1X then 1X Wash Buffer	150	2 × 30 min, RT
7. Block + immunolabel	Immunostaining Buffer (B673)	50	≥ 1 h block, then 1° / 2°
8. Wash	1X Wash Buffer (B674)	150	2 × 30 min, RT
9. RI equilibration	50% ADAPT:RI in PBS	100	≈ 30 min, RT
10. RI matching	100% ADAPT:RI (B675)	100	30 min – few hours

† Fixative not included in the kit. * Steps 5 and 6 are performed only when partial delipidation is required. Otherwise proceed from step 4 directly to step 7.

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Procedure

1. Fixation

Aspirate the culture medium from the upper edge of each well, leaving the organoid seated in the U-bottom. Add 150 µL of cold fixative per well and hold at 4 °C for up to one hour. Organoids are small; one hour is sufficient.

Do not over-fix. Excess cross-linking slows clearing and restricts antibody penetration into the organoid interior, and the effect cannot be reversed later in the workflow. Fixation chemistry also sets the ceiling on fluorescence retention: the pH 9.0 plus sucrose condition preserves sensitive endogenous reporters, and that choice is made here or not at all.

Reagent	4% PFA in PBS, pH 9.0; add 10% (w/v) sucrose for endogenous reporters (not supplied)
Volume	150 µL per well
Conditions	4 °C, up to 1 hour

2. Wash

Tilt and aspirate the fixative from the well edge, leaving 20–30 µL. Add 150 µL of 1X Wash Buffer and rock gently at room temperature for 30 minutes. Exchange the buffer and repeat once.

Reagent	1X Wash Buffer, diluted from B674 5X concentrate in PBS pH 7.4
Volume	150 µL per well
Conditions	Room temperature, gentle rocking; 2 × 30 min (1 h total)

3. Decolorization

Add 150 µL of ADAPT:DC per well. If starting in the morning, incubate over the course of the day; if starting in the afternoon or evening, incubate overnight. Check periodically — organoids are blood-free and decolorize quickly.

Reagent	ADAPT-3D Decolorization Buffer, ADAPT:DC (B671)
Volume	150 µL - 225 µL per well
Conditions	Room temperature, gentle agitation, until partial transparency

What this step does. Decolorization removes light-interfering molecules — residual pigments and autofluorescent metabolites such as NAD(P)H and flavins — and begins loosening lipids, lowering background before fluorescence imaging. Unlike whole tissue, organoids carry very little haemoglobin haem, so the value of this step in an organoid workflow is chiefly autofluorescence reduction and permeabilization ahead of RI matching. The endpoint is partial, not complete, transparency.

Pigmented models. Retinal organoids containing RPE melanin will not fully decolorize; aminoalcohol decolorants remove melanin poorly. Extend overnight, then image from the non-pigmented side or add a dedicated bleaching step.

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4. Wash

Repeat step 2: 150 µL of 1X Wash Buffer per well, 2 × 30 min at room temperature with gentle rocking. This removes spent decolorization reagent before the chemically distinct buffers that follow.

5. Partial Delipidation - Optional, Glass Vessel Only

Decision point. Skip steps 5 and 6 for most organoids below ~300 µm and for models that are not lipid-dense; proceed directly to step 7 and keep the entire workflow in the plate. Perform delipidation for organoids approaching or exceeding 500 µm, for hepatic, adipose-like, and other lipid-rich models, and whenever antibody penetration to the core has previously failed.

5a. Free the organoids from the matrix

Chill the plate on ice for 15–30 minutes to liquefy residual Matrigel. Using a pre-cut or wide-bore, pre-wetted tip, core the basement-membrane dome around each organoid and lift it out rather than pulling the organoid free. Transfer the organoids into a clean glass scintillation vial. Freeing the organoids before delipidation improves reagent access and prevents the gel from trapping lipids and antibody.

5b. Delipidate

Mix Buffers A and B 1:1 in the glass vial and incubate the organoids overnight until moderate transparency is observed. Inspect the vessel afterwards: deformed or softened plastic anywhere in the fluid path indicates leaching that will impair clearing downstream. Return the organoids to the U-bottom plate after the post-delipidation washes.

Reagent	Partial Delipidation Buffer A (B672) + Buffer B (B676), mixed 1:1
Volume	≈10 mL per pooled glass vial (5 mL of each buffer)
Conditions	Room temperature, gentle agitation, overnight, until moderate transparency
Vessel	GLASS ONLY — Buffer B corrodes polystyrene, PEG, and HDPE

6. Wash After Delipidation — Optional

For the first wash only, use 0.1X Wash Buffer diluted in Type 1 ultrapure water; rock gently for 30 minutes. Exchange for 1X Wash Buffer and repeat for a further 30 minutes (1 h total). Return the organoids to the 96-well U-bottom plate.

7. Blocking, Permeabilization, and Immunolabeling

Add 50 µL of Immunostaining Buffer per well and incubate for at least one hour at room temperature to block and permeabilize. Then dilute the primary antibody in the same buffer, add 50 µL per well, and incubate with gentle rotation. Wash, then repeat with the secondary antibody diluted in the same buffer.

Reagent	ADAPT-3D Immunostaining Buffer, 1X (B673) — blocks, permeabilizes, and carries antibody
Volume	50 µL per well (all three fills)
Conditions	Room temperature, gentle rotation

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One buffer, three jobs. The Immunostaining Buffer replaces the separate blocking, permeabilization, and antibody-dilution solutions of conventional whole-mount protocols. It contains 0.1 mM glycine, 0.167% Tween-20, 0.33% Triton X-100, 1% donkey serum, 1% alpaca serum, 1% BSA, 0.05% H₂O₂, 5% DMSO, and 0.09% azide. The two detergents permeabilize membranes; glycine quenches residual fixative-derived reactive groups; the sera and BSA block non-specific binding; DMSO promotes penetration; H₂O₂ suppresses endogenous peroxidase and pigment-derived background.

Antibody incubation times. Antibody penetration is a significant rate-limiting step in whole-mount organoid labeling. Small organoids typically label overnight; organoids of 300–500 µm may require 24–48 hours per antibody. Where penetration is marginal, lowering the concentration and extending the time is more effective than raising the concentration.

8. Wash

Repeat step 2: 150 µL of 1X Wash Buffer per well, 2 × 30 min at room temperature with gentle rocking.

9. Refractive-Index Equilibration — 50% RIM

Do not skip this step. Moving an organoid directly from an aqueous buffer into undiluted, concentrated RI solution causes osmotic shrinkage. The 50% equilibration eases the specimen across the osmotic gradient and is performed once, immediately before the full-strength step. Skipped equilibration is the single most common cause of shrunken or deformed cleared organoids.

Tip. At this stage, it is likely that the organoid is transparent and not visible to the naked eye. Use best practices to carefully not aspirate and damage the organoid.

Reagent	ADAPT:RI (B675) mixed 1:1 with PBS, pH 7.4
Volume	100 µL per well
Conditions	Room temperature, ≈30 minutes

10. Refractive-Index Matching — 100% RIM

Exchange the 50% solution for 100% ADAPT:RI and hold until the organoids are transparent. Complete transparency is reached only in this step; decolorization and delipidation produce partial transparency alone.

Transparency is a state, not a permanent modification. The index match holds only while the organoid remains immersed. On removal, the aqueous compartments re-equilibrate, the index mismatch returns, and the sample becomes turbid again. Keep organoids in 100% ADAPT:RI throughout imaging — in the plate, in a glass-bottom dish, on a mounted slide, or in a light-sheet chamber.

Reagent	Undiluted ADAPT:RI (B675)
Volume	100 µL per well
Conditions	Room temperature; 30 minutes to a few hours, until transparent
Final RI	≈ 1.50–1.51

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Recommended Incubation Times by Organoid Size

Incubation times scale with organoid diameter, density, and lipid content because every step in the workflow is diffusion-limited. The values below are recommended starting points for optimization, not validated endpoints — assess transparency and labeling quality directly and adjust.

Diameter	Fixation, 4°C	Decolorization	Delipidation	Antibody, each	50% RIM	100% RIM
< 150 µm	30–60 min	2–4 h	Skip	Overnight	15–30 min	30 min – 1 h
< 150 µm	1 h	4–8 h or overnight	Skip or overnight	Overnight	30 min	1–2 h
300–500 µm	1 h	Overnight	Overnight	24–48 h	30 min	2–4 h
> 500 µm ‡	1 h	Overnight, extend as needed	Overnight, mandatory	48 h+	30–60 min	4 h – overnight

‡ Organoids above 500 µm fall outside the validated range for this kit. They can be processed, but expect longer diffusion-limited steps, mandatory delipidation, and a greater likelihood of a necrotic core that traps antibody non-specifically. For thick tissue and whole organs, use the ADAPT-3D Tissue Clearing Kit (A630) and the tissue product insert.

Reagent Volume Guide

Volume per fill = (wells processed) × (µL per well); reagent per step = volume per fill × number of fills. Values assume one 100–500 µm organoid per well and scale linearly with well count. Prepare ≈15% extra plus 2–3 mL of reservoir dead volume for multichannel dispensing.

Step	Reagent	µL / Well	Fills	Per Full 96-Well Plate	Per 8-Well Column
Fixation †	4% PFA, pH 9.0 (not in kit)	150	1	14.4 mL	1.2 mL
Wash (Steps 2, 4, 8)	1X Wash Buffer (B674)	150	6	86.4 mL at 1X (17.3 mL of 5X)	7.2 mL at 1X
Decolorization	ADAPT:DC (B671)	150	1	14.4 mL	1.2 mL
Block + 1° + 2°	Immunostaining Buffer (B673)	50	3	14.4 mL	1.2 mL
RI equilibration (50%)	ADAPT:RI (B675), 1:1 in PBS	100	1	4.8 mL of B675	0.4 mL
RI matching (100%)	ADAPT:RI (B675)	100	1	9.6 mL of B675	0.8 mL
Delipidation *	PDL A + B, 1:1	pool	1	≈10 mL per pooled vial	—

† Fixative is not included in the kit. * Optional; performed in a glass vessel outside the plate. The step-6 wash that follows delipidation adds two further wash fills — 115.2 mL of 1X buffer per plate, or 23.0 mL of the 5X concentrate, still within the 25 mL fill supplied.

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Imaging

Cleared organoids are compatible with confocal, widefield with deconvolution, and light-sheet fluorescence microscopy. A transparent specimen held at a defined refractive index meets the input requirements of all three modality classes without re-preparation, so the choice of instrument is dictated by the experimental question rather than by the clearing chemistry.

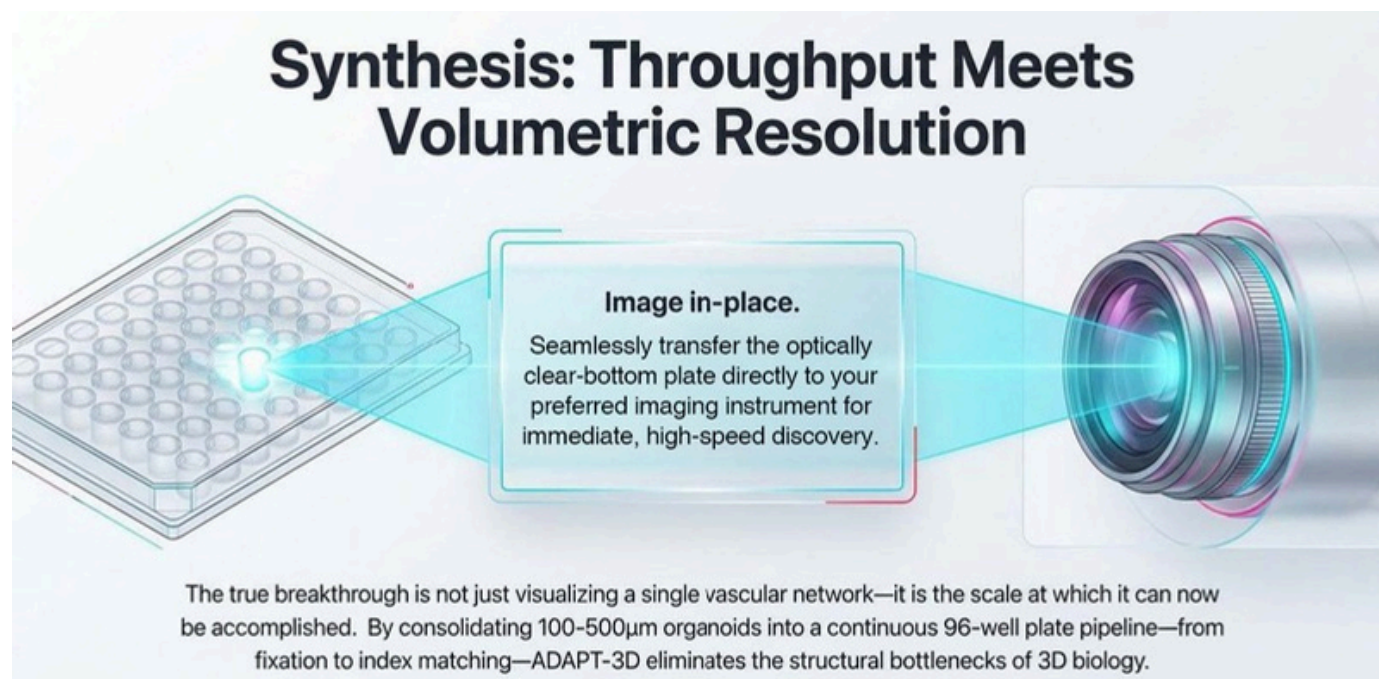


Figure 3: Image in place. Transfer the optically clear-bottom plate directly to the microscope stage. Well-to-well registration is preserved, mounting-induced distortion is eliminated, and the one-to-one correspondence between a cultured organoid and its volumetric image is retained.

- **Use an optically clear-bottom plate.** A glass-bottom or optically clear-bottom 96-well plate allows acquisition without any transfer. Seal wells with an optically clear seal to prevent evaporation during long acquisitions.
- **Keep organoids in 100% ADAPT:RI.** Transparency is lost as soon as the specimen leaves the index-matching solution.
- **Match the objective to the medium.** The final specimen index is ≈ 1.50 – 1.51 . Objectives corrected for oil or for high-index clearing media will outperform water-immersion optics at depth.
- **Manage photobleaching.** Reduce excitation power and exposure for long volumetric acquisitions; light-sheet illumination minimizes out-of-plane excitation across the volume.

Technical Support

For antibody-specific questions, unusual organoid models, or optimization support, contact Leinco Technical Support at leincoglobal@leinco.com. To search the Leinco primary antibody collection, use the Antibody Search tool at leinco.com. For thick tissue, whole organs, and sample types outside the 100–500 μm organoid range, request the ADAPT-3D Tissue Clearing Kit (A630) and its tissue product insert.

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Troubleshooting

Problem	Probable Cause	Solution
Organoid lost or damaged during processing	Organoid aspirated with the buffer	Tilt the plate and aspirate from the upper well edge, leaving 20–30 μ L. Use U-bottom plates and pre-wetted wide-bore tips.
	Shearing during transfer out of Matrigel	Use cut or wide-bore tips; core the Matrigel dome rather than pulling the organoid free.
	Cystic organoids collapse or burst	Handle gently. Keep osmotic changes gradual — always run 50% RIM before 100% RIM. Do not exceed 10% sucrose in the fixative.
Organoid remains colored or hazy after decolorization	Pigmented cell types (RPE melanin in retinal organoids; haemoglobin in erythroid or haemogenic cultures)	Extend decolorization overnight. Melanin is poorly removed by aminoalcohol decolorants; image from the non-pigmented side or add a bleaching step.
	Residual Matrigel or a dense, lipid-rich core (large, hepatic, or adipose-like organoids)	Chill the plate on ice 15–30 min and lift organoids out of the matrix. Run the glass-vial partial delipidation step.
Organoid shrinks, compresses, or deforms	50% RIM equilibration skipped	Always equilibrate $\approx$ 30 min in 50% RIM (1:1 in PBS) before 100% RIM.
	Excess sucrose in the fixative	Use 10% (w/v) sucrose. 30% sucrose is dehydrating and compresses samples.
	Organic solvent exposure	ADAPT-3D is aqueous throughout. Do not dehydrate in methanol or ethanol at any point.
Weak endogenous reporter signal (GFP, YFP, tdTomato)	Fixative at neutral pH or without sucrose	Fix in fresh 4% PFA, pH 9.0, with 10% (w/v) sucrose at 4 $^{\circ}$ C for up to 1 h. GFP/YFP-family reporters are the most pH-sensitive.
	Over-fixation	Limit fixation to 1 h at 4 $^{\circ}$ C; organoids are small and over-fixation cannot be reversed.
	Photobleaching during long volumetric acquisitions	Reduce excitation power and exposure; keep organoids in 100% RIM during imaging.
Agarose embedding of the sample failed	The organoid surface was wet when embedded	Gently blot the organoid with a lint-free wipe to minimize concentration changes from water content. Avoid over-drying.
	Agarose temperature or concentration	Use agarose at a temperature the bottle is comfortable to touch, at 1.5–4% agarose or 1% phytagel.

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Troubleshooting

Problem	Probable Cause	Solution
Weak, superficial, or patchy immunolabeling	Insufficient permeabilization and blocking	Increase block/permeabilization time in Immunostaining Buffer (B673). Add partial delipidation for organoids $\geq 500 \mu\text{m}$.
	Antibody does not penetrate a large or dense organoid	Extend antibody incubations (overnight to 2 days), lower the concentration and increase the time, and delipidate in glass.
	Residual Matrigel traps antibody	Remove organoids from the matrix before staining.
	Antibody not validated for whole-mount labeling	Verify on 2D cultures or sections first. Use IHC- or IF-validated antibodies; contact Technical Support with antibody-specific questions.
	Necrotic or dead core (common in large cerebral, tumour, and hepatic organoids)	Expect non-specific trapping in the core. Include secondary-only controls, image the viable rim, or use smaller organoids.
High background or autofluorescence	Autofluorescent metabolites and lipids retained	Complete decolorization (overnight if needed), include delipidation, and image in 100% RIM.
	Residual Matrigel or secondary antibody background	Remove residual matrix; run secondary-only and unstained controls in dedicated wells on the same plate.
Organoids did not become fully transparent	Over-fixation	Fix $\leq 1 \text{ h}$ at 4°C . Use 1–2% PFA when transparency matters more than epitope preservation.
	Incomplete decolorization or delipidation	Extend decolorization overnight; delipidate in a glass vial for large or lipid-rich organoids.
	Plastic incompatibility during delipidation	Partial Delipidation Buffer B degrades polystyrene. Use glass only; check for deformed or melted plastic, which leaches into the sample and impairs clearing.
	Organoid removed from RIM before imaging	Transparency is maintained only in 100% RIM. Image in RIM under an optically clear seal.
Wells dry out during overnight steps	Evaporation in the 96-well plate	Keep the lid on, incubate in a humidified box, and fill edge wells with PBS. Reserve rows A/H and columns 1/12 for buffer rather than samples.

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