Omni CLEAR PROTOCOL

*** Incubate at 20–25°C unless otherwise specified.*
*** Use fresh solution for each repeat. Keep the specimen fully submerged at all times and agitate on an orbital shaker at 100 rpm, and place the tube horizontally to maximize fluid flow unless otherwise specified.*

- ► Omni CLEAR is a BABB-based clearing solution.
- ► Applicable to **formaldehyde-, glutaraldehyde-,** or **polyepoxide-fixed tissues.**
- ► Use **OmniStain H&E** or **OmniStain IMMUNO** for optimal 3D staining results.
- ► For FFPE samples, use **OmniStain REZCUE** for optimal 3D antigen retrieval.

1. Place the tissue in a 50 mL conical tube.
2. Dehydrate the fixed, *delipidated*, and stained tissue in **25 mL** of 50% v/v methanol in H_2O 2 x 30 min, then in 25 mL 100% methanol 3 x 15 min.
   - Methanol quenches fluorescent proteins, but not fluorophores attached to antibodies.
   - Use adequate solvent volume during dehydration; insufficient solvent may prevent complete clearing.
3. Incubate in **20 mL** of Omni CLEAR solution for 3 hours, protected from light.
4. Replace with **20 mL** fresh Omni CLEAR solution once and incubate for at least 6 hours, or until the tissue is optically clear.
5. Proceed to imaging.
   - Omni CLEAR / BABB may dissolve or weaken some plastics, objective seals and adhesive bonding a coverslip to an imaging chamber. Prevent direct contact between Omni CLEAR / BABB and non-compatible objectives, seals, plastic parts and adhesives.
   - BABB is harmful if ingested. Avoid contact with skin and eyes. It provides the best clearing results even for the most challenging tissues.
   - The tissue maybe stored at 4 - 25°C for 3 months. Fluorescence signal may degrade over time.

For more information, scan the QR code or visit
https://illumos.co/why-do-we-use-babb-all-the-time

Have questions? Contact us at sales@illumos.co or visit www.illumos.co

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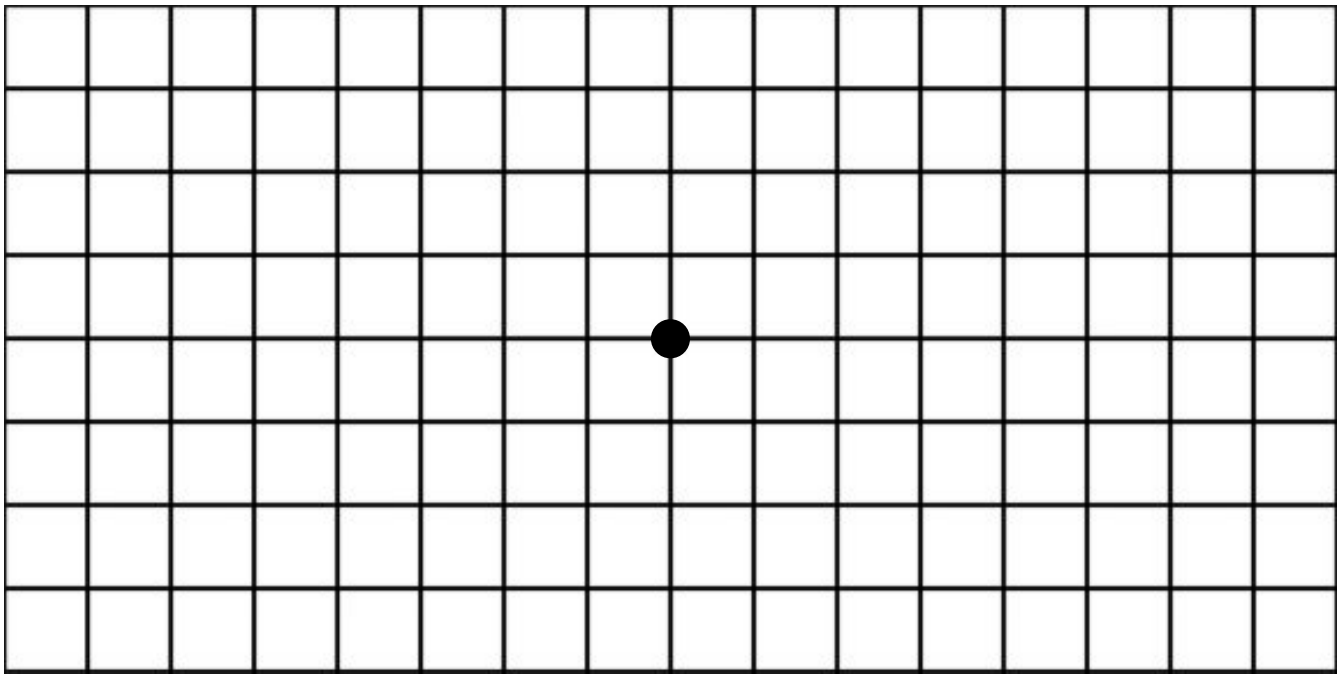
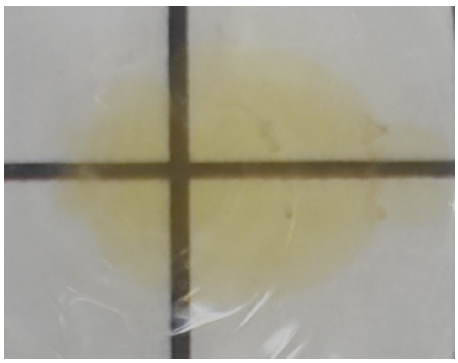
► To assess whether the tissue is sufficiently clear for imaging, transfer it to a clear glass or quartz cuvette and position it over the grid below.

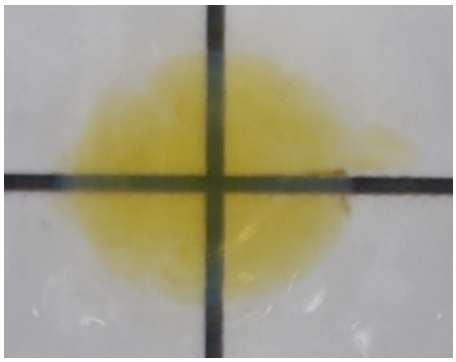


Good clearing

Best

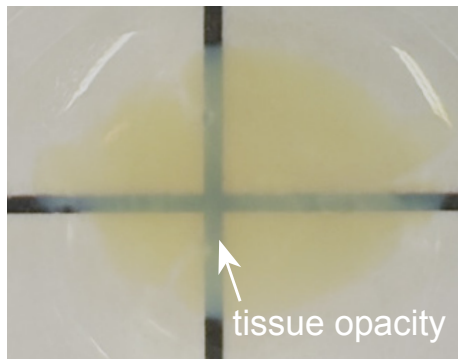


Good



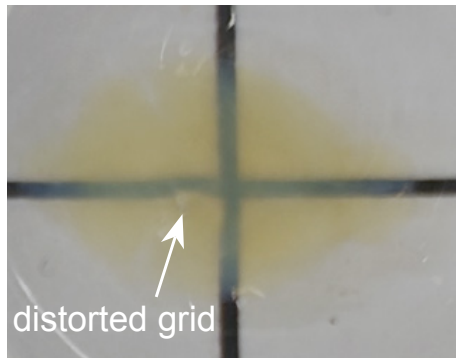
Bad clearing

Insufficient dehydration / delipidation



Light scatterers may result from microscopic refractive index inhomogeneities, usually due to retained moisture or inadequate tissue delipidation. Rehydrate the tissue in 50% methanol, and repeat the dehydration and delipidation step with longer incubation time. Re-staining may be required.

Inadequate clearing time / volume



Distorted grid lines may result from a mismatch between the refractive index of the tissue and that of the surrounding Omni CLEAR. Refresh the Omni CLEAR solution and increase the incubation volume to ensure they reach the same final refractive index.

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