OmniStain REZCUE 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.*

TISSUE PREPARATION **use a fume hood and place the bottle upright during shaking for Steps 1-5.

1. Remove excess paraffin with a scalpel without cracking the tissue. Place the tissue into a 100 mL GL45 laboratory glass bottle.
2. Incubate in 25 mL methylcyclopentane (4 x 1 hour) to dissolve most of the paraffin.
3. Replace with 50 mL cyclohexane and incubate for 6-18 hours.
4. Replace with 50 mL fresh cyclohexane and incubate for 1 h.
5. Replace with 50 mL 85:15 v/v THF:methanol for 6-18 hours.
6. Transfer the tissue to a new 50 mL conical tube. Rehydrate sequentially in 25 mL 100% methanol (3 x 30 min), 25 mL 50% v/v methanol in H_2O (2 x 30 min), and 25 mL ddH_2O (2 x 30 min). Shake the tube horizontally.

ANTIGEN RETRIEVAL

7. Pre-warm aliquots of **1x OmniStain REZCUE reagents A, B, and C** and ddH_2O to 55°C.
8. For each tissue, add the following components in the order shown (total 10 mL per tissue):
   - 1.5 mL ddH_2O
   - 5 mL **1x OmniStain REZCUE reagent A**
   - 1 mL **1x OmniStain REZCUE reagent B**
   - 2.5 mL **1x OmniStain REZCUE reagent C**
9. Upon cooling, the mixture may form a white precipitate. Warm the closed tube to 55°C until the precipitate redissolves completely; this may take up to 1 hour.
10. Agitate the tube upright at 55°C for at least 12 hours, *but not longer than 48 hours in total.*
    - Pre-warm at least 50 mL ddH_2O alongside each tissue for the two 25 mL washes in Step 11.
    - If precipitates appear, increase the temperature to 60°C for no more than 2 hours total. Once dissolved, return to 55°C.
    - Air incubators may equilibrate slowly. Use a water or metal-bead bath for better temperature control.

WASHING

11. Wash the tissue in 25 mL ddH_2O at 55°C (2 x 1 hour).
12. Wash the tissue in 25 mL 50% v/v DMSO in H_2O at room temperature (1 x 2 hours).
13. Wash the tissue in 25 mL ddH_2O (2 x 30 min). The tissue may proceed to validated workflows for formalin-fixed tissue, or be stored in 1x PBS with 0.02% w/v sodium azide at 4°C for up to 1 year.
14. Proceed to **OmniStain H&E** or **OmniStain IMMUNO** for general morphological staining or multiplexed immunostaining, respectively.

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

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OmniStain REZCUE PROTOCOL

Introduction

- **OmniStain REZCUE** is an **antigen retrieval** method for 3D multiplexed immunostaining of FFPE tissue, designed to preserve tissue morphology and molecular analytes.
- Supports deep and uniform penetration of compatible dyes and antibodies, and prepares FFPE tissue for solvent-based tissue clearing.
- **Typical processing time is 3-4 days** for standard pathology tissue blocks (3 cm x 2 cm x 5 mm), without specialized equipment.
- Compatible with downstream 3D H&E staining using **OmniStain H&E** (takes an additional 24 hours).
- Compatible with 3D multiplex immunostaining using **OmniStain IMMUNO** (takes an additional 8-10 days).
- After **OmniStain REZCUE**, **OmniStain H&E** plus **OmniStain IMMUNO**, the sample can be used for:

1. Routine 2D H&E, special stains. (For subsequent routine 2D IHC, use the H&E-only workflow; do not perform OmniStain IMMUNO beforehand).
2. **Whole-genome/exome DNA sequencing**
3. Bulk transcriptomics by **RNA sequencing**
4. FISH-based **spatial transcriptomics**
5. Mass spectrometry-based **spatial proteomics**

Materials provided

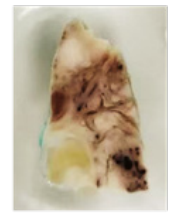
- **1x OmniStain REZCUE reagent A**
- **1x OmniStain REZCUE reagent B**
- **1x OmniStain REZCUE reagent C**

Materials not provided but required

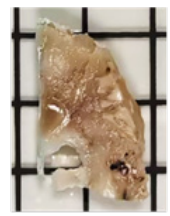
- 55-60°C shaking incubator
- Methylcyclopentane
- Cyclohexane
- Tetrahydrofuran (THF), stabilized or unstabilized
- DMSO
- Methanol
- ddH_2O
- Fume hood
- 100 mL GL45 laboratory glass bottles
- 50 mL conical tubes (made of polypropylene)

Example (human breast cancer)

FFPE block

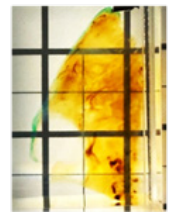


Deparaffinized
After step 4

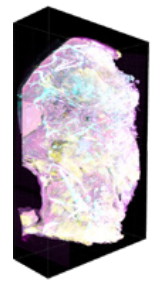


Antigen-retrieved
After step 13

3D mIHC
OmniStain IMMUNO

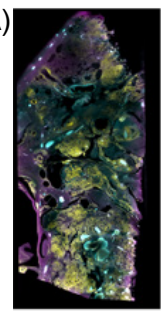


Omni CLEAR

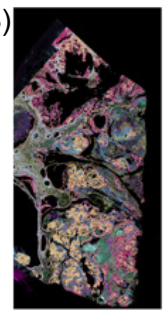


Light-sheet microscopy imaging

(A)



(B)



(C)

(A) 4-plex 3D IHC

(B) Spatial transcriptomics (6,000 genes)

(C) 2D H&E
+ Spatially referenced MS-proteomics (>7,000 proteins)
+ Spatially referenced mRNA sequencing
+ *En bloc* whole-exome sequencing

**Results depend on the platform, specimen, input quality and analysis method.

More resources at:
www.illumos.co/a-quick-guide-to-omnistain/