



VERSION 2

APR 16, 2024

OPEN ACCESS



DOI:

[dx.doi.org/10.17504/protocols.io.e6nvw16p7lmk/v2](https://dx.doi.org/10.17504/protocols.io.e6nvw16p7lmk/v2)

**Protocol Citation:** Kyu Sang Han, Pei-Hsun Wu, Joel Sunshine, Ashley Kiemen, Sashank Reddy, Denis Wirtz 2024. Tissue Harvesting | HuBMAP | JHU-TMC.

protocols.io

<https://dx.doi.org/10.17504/protocols.io.e6nvw16p7lmk/v2>Version

created by [Kyu Sang Han](#)

## Tissue Harvesting | HuBMAP | JHU-TMC V.2

Kyu Sang Han<sup>1</sup>, Pei-Hsun Wu<sup>1</sup>, Joel Sunshine<sup>2</sup>, Ashley Kiemen<sup>2</sup>, Sashank Reddy<sup>2</sup>, Denis Wirtz<sup>1,2</sup>

<sup>1</sup>Johns Hopkins University; <sup>2</sup>Johns Hopkins Medicine

Human BioMolecular Atlas Program (HuBMAP) Method Development Community

TMC - Johns Hopkins University



Kyu Sang Han

Johns Hopkins University

### DISCLAIMER

The [protocols.io](#) team notes that research involving animals and humans must be conducted according to internationally-accepted standards and should always have prior approval from an Institutional Ethics Committee or Board.

### ABSTRACT

This protocol describes how to harvest human tissue biopsy and prepare it for histological processes

### PROTOCOL REFERENCES

Khoury T, Sait S, Hwang H, Chandrasekhar R, Wilding G, Tan D, Kulkarni S. Delay to formalin fixation effect on breast biomarkers. Mod Pathol. 2009 Nov;22(11):1457-67. doi: 10.1038/modpathol.2009.117. Epub 2009 Sep 4. PMID: 19734848.

Bauer DR, Stevens B, Chafin D, Theiss AP, Otter M. Active monitoring of formaldehyde diffusion into histological tissues with digital acoustic interferometry. J Med Imaging (Bellingham). 2016 Jan;3(1):017002. doi: 10.1117/1.JMI.3.1.017002. Epub 2016 Feb 8. PMID: 26866049; PMCID: PMC4744337.

Bonnie Gambichler, Alan Meeker, "Tissue Handling Procedures", 28 March 2024, [tmalab.jhmi.edu/histology.html#proc](https://tmalab.jhmi.edu/histology.html#proc)

**MANUSCRIPT CITATION:**

A.M. Braxton, A.L. Kiemen, M.P. Grahn, A. Forjaz, J. Parksong, J.M. Babu, J. Lai, L. Zheng, N. Niknafs, L. Jiang, H. Cheng, Q. Song, R. Reichel, S. Graham, A.I. Damanakis, C.G. Fischer, S. Mou, C. Metz, J. Granger, X.-D. Liu, N. Bachmann, Y. Zhu, Y.Z. Liu, C. Almagro-Pérez, A.C. Jiang, J. Yoo, B. Kim, S. Du, E. Foster, J.Y. Hsu, P.A. Rivera, L.C. Chu, D. Liu, E.K. Fishman, A. Yuille, N.J. Roberts, E.D. Thompson, R.B. Scharpf, T.C. Cornish, Y. Jiao, R. Karchin, R.H. Hruban, P.-H. Wu, D. Wirtz, and L.D. Wood, “3D genomic mapping reveals multifocality of human pancreatic precancers”, *Nature* (2024)

A.L. Kiemen, A. Forjaz, R. Sousa, K. Sang Han, R.H. Hruban, L.D. Wood, P.H. Wu, and D. Wirtz, “High-resolution 3D printing of pancreatic ductal microanatomy enabled by serial histology”, *Advanced Materials Technologies* 9, 2301837 (2024)

T. Yoshizawa, J. W. Lee, S.-M. Hong, D.J. Jung, M. Noe, W. Sbijewski, A. Kiemen, P.H. Wu, D. Wirtz, R.H. Hruban, L.D. Wood, and K. Oshima. “Three-dimensional analysis of ductular reactions and their correlation with liver regeneration and fibrosis”, *Virchows Archiv* (2023).

A.L. Kiemen, A.I. Damanakis, A.M. Braxton, J. He, D. Laheru, E.K. Fishman, P. Chames, C. Almagro Perez, P.-H. Wu, D. Wirtz, L.D. Wood, and R. Hruban, “Tissue clearing and 3D reconstruction of digitized, serially sectioned slides provide novel insights into pancreatic cancer”, *Med* 4, 75-91 (2023)

A. Kiemen, Y. Choi, A. Braxton, C. Almagro Perez, S. Graham, M. Grahm, N., N. Roberts, L. Wood, P. Wu, R. Hruban, and D. Wirtz, “Intraparenchymal metastases as a cause for local recurrence of pancreatic cancer”, *Histopathology* 82: 504-506 (2023)

(2022)

A.L. Kiemen, A.M. Braxton, M.P. Grahm, K.S. Han, J.M. Babu, R. Reichel, A.C. Jiang, B. Kim, J. Hsu, F. Amoa, S. Reddy, S.-M. Hong, T.C. Cornish, E.D. Thompson, P. Huang, L.D. Wood, R.H. Hruban, D. Wirtz and P.H. Wu, "CODA: quantitative 3D reconstruction of large tissues at cellular resolution", Nature Methods 19: 1490-1499 (2022)

K.S.Han, I. Sander, J. Kumer, E. Resnick, C. Booth, B. Starich, J. Walston, A.L. Kiemen, S. Reddy, C. Joshi, J. Sunshine, D. Wirtz, P.-H. Wu "The microanatomy of human skin in aging." bioRxiv (2024): 2024-04.

**License:** This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

**Protocol status:** Working

We use this protocol and it's working

**Created:** Apr 16, 2024

**Last Modified:** Apr 16, 2024

**PROTOCOL integer ID:** 98295

**Funders Acknowledgement:**

Institute of Arthritis and Musculoskeletal and Skin Diseases

Grant ID: U54AR081774

National Cancer Institute

Grant ID: U54CA143868

## Prepare tissue collection container

- 1 For tissue collection container, we will prefill histology containers with 10% Neutral Buffered Formalin (NBF)

- 2 Combine 100 mL of Formaldehyde (37% - 40%), 900mL of distilled water, 4g of Sodium dihydrogen phosphate monohydrate, and 6.5g of disodium hydrogen phosphate anhydrous.
- 3 Confirm that the pH of the solution is between 6.8 and 7.2.
- 4 Fill the solution into histology containers
- 5 Store at room temperature until use.
- 6 One can purchase this instead: VWR® Prefilled Histology Containers, 10% Neutral Buffered Formalin (NBF) Cat. No. 16004-121

## Biopsy - scalp

- 7 The surgical procedures for scalp skin include tissue expansion, nerve release, hair transplantation, local tissue rearrangement.
- 8 Hair bearing areas are chosen and areas with a paucity of hair follicles including those from advanced androgenic alopecia or alopecia areata are excluded.

## Biopsy - trunk

- 9 We collect our samples from tissues normally discarded during surgical procedures. The surgical procedures for trunk skin include deep inferior epigastric artery perforator flaps, panniculectomy, abdominoplasty, reduction mammoplasty.

## Fixation

- 10 Once the biopsy is harvested, we immediately put it into the tissue collection container to fix the tissue and avoid the degradation. The time delay between tissue excision and fixation, warm ischemic time, is a critical factor.
- Note on the effect of delay to formalin fixation - [Delay to formalin fixation effect on breast biomarkers - PubMed \(nih.gov\)](#)
- 11 Please refer to our [tissue fixation protocol](#) for the next steps