Apr 16, 2024 Version 2

Tissue Fixation | HubMAP | JHU-TMC V.2

DOI
dx.doi.org/10.17504/protocols.io.3byl4929jgo5/v2

Kyu Sang Han[1], Pei-Hsun Wu[1], Joel Sunshine[2], Ashley Kiemen[2], Miklhail James[2], Sashank Reddy[2], Denis Wirtz[1,2]

[1]Johns Hopkins University; [2]Johns Hopkins Medicine

Human BioMolecular Atlas Program (HuBMAP) Method Development Community
Tech. support email: **Jeff.spraggins@vanderbilt.edu**

Kyu Sang Han
Johns Hopkins University

OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.3byl4929jgo5/v2

Protocol Citation: Kyu Sang Han, Pei-Hsun Wu, Joel Sunshine, Ashley Kiemen, Miklhail James, Sashank Reddy, Denis Wirtz 2024. Tissue Fixation | HubMAP | JHU-TMC. **protocols.io https://dx.doi.org/10.17504/protocols.io.3byl4929jgo5/v2**Version created by **Kyu Sang Han**

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 (2022)

A.L. Kiemen, A.M. Braxton, M.P. Grahn, 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. Joshu, J. Sunshine, D. Wirtz, P.-H. Wu "The microanatomy of human skin in aging." bioRxiv (2024): 2024-04.

Protocol status: Working
We use this protocol and it's working

Created: April 16, 2024

Last Modified: April 16, 2024

Protocol Integer ID: 98294

Funders Acknowledgements:
Institute of Arthritis and Musculoskeletal and Skin Diseases
Grant ID: U54AR081774
National Cancer Institute
Grant ID: U54CA143868

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

First and most important - the original tissue sample must be of good quality. Factors such as warm ischemic time, the time delay between tissue excision and fixation, etc. are important. Ideally, tissues should be acquired as close to still being viable as possible, and put into fixative as soon as possible following excision. Delays lead to cell death, autolysis, and loss of tissue and cell integrity with concomitant losses of immunostaining (e.g., due to proteolysis of the antigen). If acquiring animal tissues, consider performing perfusion fixation before organ/tissue removal if it is an option

Tissue Fixation

1 Tissue is harvested using our protocol (**Tissue Harvesting Protocol**)

2 We submerge the biopsy in the histology container prefilled with 10% NBF for fixation

3 Minimizing the warm ischemic time is critical. The time delay between tissue excision and fixation is detrimental to further analysis.

Note on the effect of delay to formalin fixation - **Delay to formalin fixation effect on breast biomarkers - PubMed (nih.gov)**

4 The rate of penetration of formaldehyde depends on the size of the biopsy. Trimming is often required to facilitate fixation. Tissues placed in the tissue cassettes should be no thicker than 3-4mm because the interior of a specimen may not become fully fixed, or significant autolysis can occur.

Reference - **Oncology Tissue and Imaging Services – at Johns Hopkins (jhu.edu)**

Tissue Trimming

5 Trimming also helps remove unwanted parts of the biopsy such as excessive fat and traumatized
edges.

6 Label tissue cassette with sample ID with pencil

7 Place the trimmed tissue in cassette

8 Place cassettes back into the histology container

Tissue fixation (continue)

9 Refill 10% NBF into the container and leave for 12~48 hours at room temperature.

Use plenty of fixative. The general rule is to use at least 15 volume equivalents of formalin per volume
of tissue. A higher formalin-to-tissue ratio certainly won't hurt, and just requires a larger container.
Formalin is relatively cheap, so don’t skimp on this step.

Recommended reading - **Active monitoring of formaldehyde diffusion into histological tissues with digital acoustic interferometry - PMC (nih.gov)**

10 Gentle agitation of the tissue in the formalin during fixation maximize diffusion and reduce the poor local fixation.

11 Pour the formalin into an “excess formalin” waste container in the hood.

12 Rince the cassettes in the histology container with 1x PBS

13 Drain and refill the PBS. Leave the cassette submerged in PBS and store at 4 degree C until processing.

14 Properly fixed tissue can be stored in PBS up to a week.

Protocol references

Oncology Tissue and Imaging Services – at Johns Hopkins (jhu.edu)