

Resource Summary Report

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Whole-body tissue stabilization and selective extractions via tissue-hydrogel hybrids for high-resolution intact circuit mapping and phenotyping

DOI:10.17504/protocols.io.8xehxje

Type: Protocol

Proper Citation

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Protocol Information

URL: <https://dx.doi.org/10.17504/protocols.io.8xehxje>

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Group: Optical Clearing of Tissue, Neurodegeneration Method Development Community

Summary: To facilitate fine-scale phenotyping of whole specimens, we describe here a set of tissue fixation-embedding, detergent-clearing and staining protocols that can be used to transform excised organs and whole organisms into optically transparent samples within 1–2 weeks without compromising their cellular architecture or endogenous fluorescence. PACT (passive CLARITY technique) and PARS (perfusion-assisted agent release in situ) use tissue-hydrogel hybrids to stabilize tissue biomolecules during selective lipid extraction, resulting in enhanced clearing efficiency and sample integrity. Furthermore, the macromolecule permeability of PACT- and PARS-processed tissue hybrids supports the diffusion of immunolabels throughout intact tissue, whereas RIMS (refractive index matching solution) grants high-resolution imaging at depth by further reducing light scattering in cleared and uncleared samples alike. These methods are adaptable to difficult-to-image tissues, such as bone (PACT-deCAL), and to magnified single-cell visualization (ePACT). Together, these protocols and solutions enable phenotyping of subcellular components and tracing cellular connectivity in intact biological networks.

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External URL: <https://www.nature.com/articles/nprot.2015.122>

Version: 1

Publication Date: 2019

Proper Citation: Jennifer B Treweek, Ken Y Chan, Nicholas C Flytzanis, Bin Yang, Benjamin E Deverman, Alon Greenbaum, Antti Lignell, Cheng Xiao, Long Cai, Mark S Ladinsky, Pamela J Bjorkman, Charless C Fowlkes, Viviana Gradinaru 2019. Whole-body tissue stabilization and selective extractions via tissue-hydrogel hybrids for high-resolution intact circuit mapping and phenotyping. protocols.io
dx.doi.org/10.17504/protocols.io.8xehxje

Record Creation Time: 20210329T220526+0000

Record Last Update: 20210329T220921+0000

Data and Source Information

Source: