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Organoid Immunohistochemistry on Ventana Benchmark XT

Forked from a private protocol

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Organoid and Assembloid



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Protocol status: Working

We use this protocol and it's working

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Abstract

Organoid Immunohistochemistry on Ventana Benchmark XT

Immunohistochemistry of organoid sections

- 1 Fix organoids in 10% NBF for 15-30 minutes.
- 2 Prepare paraffin-embedded blocks on tissue processor.
- 3 Cut 5µm sections from FFPE (formalin-fixed paraffin-embedded) blocks using microtome.
- 4 Mount sections on positively charged slides and bake overnight at 70° C.
- 5 Prepare primary antibody dilution kit (depending on antibody concentration) for Ventana Benchmark XT automatic staining platform (Roche Diagnostics, Indianapolis, IN) according to the manufacturer's protocol.
- 6 Load slides onto Ventana and run through alcohol, antigen retrieval, primary antibody incubation and visualization steps.
- 7 Coverslip slides and let dry overnight.

Digital Histopathologic Analysis in QuPath

- 8 Scan slides at 40x on Aperio VERSA 8 (Leica Biosystems, Wetzlar Germany) digital slide scanner.
- 9 Manually segment organoids to generate annotations in QuPath (version 0.2.3, <https://qupath.github.io/>).
- 10 Perform color deconvolution and determine appropriate settings for positive-pixel-based analysis.