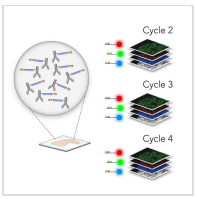

May 24, 2022

CODEX® Multiplexed Imaging | Modality Overview

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Vanderbilt Diabetes Research Center

Islet and Pancreas Analysis Core
Vanderbilt Diabetes Research Center

This is an overview of all protocols currently in use by the Vanderbilt Diabetes Research Center Islet & Pancreas Analysis (IPA) Core and Powers/Brissova Research Group for performing multiplexed imaging of the human pancreas using the CO-Detection by indEXing (CODEX®) platform (now PhenoCycler™; Akoya Biosciences). It includes links to each of the individual protocols that make up the workflow for various applications and projects.

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protocol

Walker JT, Saunders DC, Rai V et al. RFX6-mediated dysregulation defines human β cell dysfunction in early type 2 diabetes. Biorxiv (2021) doi:10.1101/2021.12.16.466282.

protocol ,

Mar 25, 2022

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Mar 25, 2022 Diane Saunders Vanderbilt University

May 10, 2022 Islet and Pancreas Analysis Core Vanderbilt Diabetes Research Center

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1 Procure and/or prepare samples fixed with 4% paraformaldehyde (PFA).

HuBMAP | VU TMC Eye/Pancreas | Human Pancreas Processing for Multiple Applications (HuBMAP)
Mouse Pancreas Dissection and Fixation for Cryosectioning (IPA Core)

4% PFA fixation optimally preserves pancreas cell morphology and improves staining quality. Tissues may be embedded in either Optimal Temperature Cutting (OCT) media or carboxymethylcellulose (CMC).

2 Prepare coverslips with pancreas sections.

CODEX® Multiplexed Imaging | Tissue Sectioning
by Islet and Pancreas Analysis Core, Vanderbilt Diabetes Research Center

PREVIEW RUN

ⓘ Samples mounted on coverslips can be stored at **-80 °C** for up to 2 months. All of our optimization of this protocol was done on **10 µm** cryosections.

3 Design antibody panel using a combination of preconjugated or custom-conjugated primary antibodies.

CODEX® Multiplexed Imaging | Antibody Conjugation and Validation
by Islet and Pancreas Analysis Core, Vanderbilt Diabetes Research Center

PREVIEW RUN

ⓘ For information on HuBMAP Organ Mapping Antibody Panels (OMAPs), please visit the CCF Portal here.

4 Label tissue with primary antibodies and prepare reagents for imaging.

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CODEX® Multiplexed Imaging | Tissue Staining and Reporter Plate Preparation
by Islet and Pancreas Analysis Core, Vanderbilt Diabetes Research Center

PREVIEW RUN

ⓘ See also: Akoya Biosciences PhenoCycler™ User Manual

5 Perform cyclic fluorescent imaging of tissue.

CODEX® Multiplexed Imaging | Microscope Setup and Tissue Imaging
by Islet and Pancreas Analysis Core, Vanderbilt Diabetes Research Center

PREVIEW RUN

6 Process raw imaging data.

As of May 2022, raw imaging data is processed using CODEX Processor v1.8.1.9 (Akoya Biosciences), which performs image stitching, z-plane selection, background subtraction, deconvolution, and shading correction.

ⓘ See also: CODEX® Processor User Instructions & Technical Notes

7 [Optional] Perform registration with other imaging modalities.

Autofluorescence (AF) Registration (HuBMAP)

Data Processing and Image Registration
by Jamie Allen, Vanderbilt University

PREVIEW RUN

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