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CODEX Acquisition Protocol

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1 Works for me dx.doi.org/10.17504/protocols.io.bdifi4bn

Human BioMolecular Atlas Program (HuBMAP) Method Development Community

ABSTRACT

Multiplex imaging of lymph node, thymus and spleen is accomplished using the Akoya Biosciences CODEX system at the HubMAP Tissue Mapping Center at the University of Florida, TMC-UF.

This protocol is an overview of the CODEX raw data acquisition process guided by the CODEX Instrument Manager (CIM) , which is part of the CODEX Software Suite. The CODEX Instrument Manager is necessary to perform CODEX experiments. It controls the fluidics of the CODEX instrument the integration and synchronization with the Keyence microscope, image data formatting and facilitates transfer of data to the analysis computer.

For more information, consult the Akoya Biosciences CODEX User Manual - A.0.

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

CODEX User Manual-REV A.0.© 2019 by Akoya Biosciences, Inc.

GUIDELINES

Imageable tissue thickness: ≤10 µm
Maximum biomarker capacity: Up to 35 cycles (or 105 markers)

MATERIALS

NAME	CATALOG #	VENDOR
KimWipes		Fischer Scientific
DMSO	BP231	Fisher Scientific
Premium Microcentrifuge Tubes: 1.5mL, Amber; 1.5mL; O.D. x L: 10.8 x 40.6mm	05408134	Thermo Fisher
10X Codex Buffer	232119	Akoya Biosciences
Codex Gaskets	7000004	Akoya Biosciences
EMS Glass Cover Slips 22 mm X 22 mm #1 1/2	72204-01	Electron Microscopy Sciences
Nuclear Stain CODEX Reagent		Akoya Biosciences
Dumont #5/45 - Cover Slip Forceps	11251-33	Fine Science Tools
50 ml Beaker		Fisher Scientific

MATERIALS TEXT

Distilled water or Milli-Q ultrapure water

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SAFETY WARNINGS

DMSO is readily absorbed through the skin. Wear nitrile gloves when handling. Dispose of waste according to local regulations.

BEFORE STARTING

- Allow for 96-well plate containing the Reporter Master Mix solutions and antibody stained-tissue sections to equilibriate to room temperature for a minimum of 15 minutes.
- Dilute sufficient 10X CODEX buffer to 1X CODEX buffer 1:10 with ddH2O to complete the number of cycles in the experiment according to the chart below. Fill CODEX instrument bottles with 1X codex buffer, ddH2O and DMSO.

Number of Cycles*	10x CODEX Buffer	ddH2O	1x CODEX Buffer	DMSO
[ml]	[ml]	[ml]	[ml]	
5	31	281	312	236
8	44	394	438	318
10	53.5	480.5	534	386
12	63.5	570.5	634	454
15	78.5	705.5	784	556
18	93.5	840.5	934	658
20	103.5	930.5	1034	726
25	128.5	1155.5	1284	896

Required Reagent Volume by Cycle Number

Set up CODEX experiment

1 Quick Start Experiment Setup: Turn on CIM computer and CODEX robot

1.1 Launch Codex Instrument Management Software.

1.2 Select the **Experiment** Tab to start the run.

1.3 Select **New Template** for inputting experimental settings from scratch or **Open Template** to modify an exiting template,

1.4 Input the Project and Experiment name.
Use the naming format of: src_CX_<lab-caseID>_<tissue-id>_<block-id>_<DATE[MMDDYY]>

1.5 Input the Start Cycle Well and Number of Cycles in agreement with the 96-well reporter plate to be used for this experiment.

1.6 Within the table, for each cycle, enter the Marker Name, Exposure time, and Class. Marker names should follow the convention agreed to by the HubMap Consortium

The first and last cycles contain nuclear stain only and are labled **BLANK for both marker and class**. Any unused wells (no marker) are labeled **EMPTY for both marker and class**.

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1.7 Select the number of Z-Planes to image. For a 5um section, 11 planes are standard.
An uneven section, large region of acquisition or thicker section all require increases in number of Z-planes.
The software will alter this number based on the formula provided below the input box, resulting in a number that is less than the set.point

1.8 Enter the **Operator Name.**

1.9 **Click Validate Experiment** to confirm there are no errors within the experimental design.

1.10 Press **Save Template** or **Save Template As** to save the experimental setting if needed, **Save Template As** can be used to avoid over writing previous settings.

2 Setup the CODEX instrument with the start-up Wizard

2.1 Press **Start Experiment.**

2.2 The initial pop-up will describe each of the steps used in the Wizard. Press **Next.**

loading a blank coverslip and priming the instrument

2.3 Fill the water bottle with distilled water, at least 100mL.
Fill bottle 2 with DMSO and fill Bottle 1 with 1x CODEX buffer according to the set-up chart in **Guidelines &Warnings**. Make sure to reconnect all lines and to firm all caps on the corresponding bottles.

2.4 Check that the waste bottle is empty and the 4 buffer reservoirs located inside the instrument are empty and clean.

2.5 Prepare Stage Insert for priming the instrument. Follow the steps below to load a **blank** coverslip.

3 :Untwist the two wing knobs on the coverslip stage to remove the lid holding the fluidic line

3.1 If running 2 experiments back to back, first remove old cover slip, using bent angle forceps. Rinse area well with deionized water. Wipe dry with kimwipe.

3.2 Soak two gaskets in 1X CODEX buffer for at least 10 minutes.

3.3 Place the first gasket into the divots at the center of the stage. Tap gently with forceps to be sure it is well seated. Place a clean coverslip (required for priming) on top of the first gasket. If a sample coverslip is inserted, confirm that the tissue is facing upwards. Tap the edges of the coverslip to make sure it is adhered to the first gasket. Quickly place the second gasket on top of the coverslip.

3.4 Check that the gaskets and coverslip are inserted squarely into the stage. Place the metal top plate on the stage insert and lock it in place by turning the wing knobs.

3.5 Place loaded stage insert into dedicated holder to support it and keep it level during priming.

4 After setting up the stage with a **blank** coverslip, Click **next** to initiate the start-up wizard.

4.1 Click **Check Fluidics.**

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4.2 Select **next.** The wizard will prompt you to Prime Instrument, click **Prime Instrument** to initiate procedure.

Load and DAPI stain sample coverslip

5 The wizard will prompt performing Nuclear Staining of tissue on coverslip.

5.1 Remove DAPI Nuclear Staining stock solution from 4C storage and place it on ice.

5.2 Label a 1.5ml amber microfuge tube for Nuclear Stain Solution. Add 1 mL of 1x CODEX Buffer and 0.5ul of DAPI, invert to mix.

5.3 Remove the blank coverslip and load a sample coverslip in to the stage insert as in step 3.4.. Quickly add 700uL of the Nuclear Stain Solution to the sample well and protect it from light.

Be sure that the sample coverslip is face up in the stage insert. Avoid pipetting directly on the tissue. Do not allow the tissue to dry out. Use a moist kim wipe to clean the underside of the sample coverslip *after* completing the nuclear stain, but before placing the stage insert into the microscope.
Dry thoroughly.

5.4 Press **Wait & Wash Tissue.** The instrument will perform a 5 minute incubation before washing the sample well.

If the 5 minutes incubation time has passd, click **Immediately Wash Tissue** to remove the Nuclear Stain solution.

Microscope set up start acquisition

6 The wizard will prompt the microscope set up.
Turn on the power supply to the BZ-X800 Keyence microscope. Turn on the microscope.

6.1 Open the microscope viewer software and login with lab designated PassWord.

6.2 Remove the 10x and 40x objectives from the microscope. Set the microscope to have the 20x objective in position for use.
Place the liquid overflow detector in the objective turret with the cord trailing from the left side stage cut out.

6.3 Confirm placement of fluorescent filter cubes:
DAPI in position 1, AF750 in position 2, ATTO550 in position 3, and Cy5 in position 4.

6.4 Confirm that the saved settings are compatible with the Codex software:
Stage holder= versatile
Multichannel on, all four channels set to white color and active
High resolution, low photobleaching, Monocromatic, Stitching, Z-stacking all "ON"
Set capture area to 7X9, pitch to 1.5, number of Z-planes to match number calculated in step 1.7.

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6.5 Create the folder for collecting and storing the raw images.
Available folders are preloaded in the lab share drive "CODEX Runs" folder.
Identify the specific data set using the experimental name designated in step 1.4.

6.6 Place the Stage Insert into the microscope stage holder. Activate the DAPI channel and auto focus on the center of the tissue sample.

6.7 Use the Navigation function of the microscope to select imaging region(s). Capture an image of the stitch area and save it to the folder created for this data set. Confirm that the image routed to the correct folder.

6.8 Set the z-plane minimum and maximum values. The exact planes used for each tile will be determined through auto focus by the CIM driver software during image acquisition.

Confirm the pitch is set to 1.5 and define a stitch area (i.e. 9X9). Set the Z-planes so that the best focus is the middle point of the Z-stack. Check multiple areas of the image area to be sure that the Z-stack has sufficient depth to capture the best focus over the entire image.

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6.9 Leave the microscope window open and on full view. Failure to do so may result in the driver software shutting down.

6.10 Switch back to the CODEX wizard software, select **Microscope Pre-Check.** Once it passes assessment, **Start** the run

6.11 When run is complete, confirm there are no errors in the error log.

6.12 Close the microscope software and power off the microscope.

Post Run Clean Instrument Wash

7 Post run wash.

This should be performed immediately following every CODEX run. It will take approximately 5 minutes to complete.

7.1 Remove the stage insert from the microscope and place it in the dedicated holder. Make sure that the Water Bottle has at least of 20mL of distilled water. Run **Clean Instrument Wash** from the Maintenance Tab. When the wash run is completed, remove the 96-well plate, empty and clean the reservoirs and all bottles. Close the Driver software and turn off the CODEX instrument. **00:05:00**

DMSO is present in all reservoirs and in the waste bottle, dispose of liquids properly.

Maintenance Wash

8 Maintenance Wash

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8.1 Maintenance Wash should be performed Weekly during daily use of the instrument or Monthly for less frequent use of the instrument.
A blank coverslip needs to be in the stage insert. Both Bottle 1 and Bottle 2 caps are placed in the water bottle. This will ensure full cleaning of all lines.

8.2 Select **Maintenance Wash** from the **Maintenance** tab.

8.3 When the Maintenance wash is completed return the fluid lines to their respective bottles. Remove and discard the coverslip. Wash the gaskets the stage insert with distilled water. Store them dry and covered with a kim wipe to avoid dust.

8.4 Empty waste bottle.
Turn off robot.
Close CODEX driver software and power off the CIM computer.

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