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## CODEX Preparation of Reporter 96-Well Plates

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Human BioMolecular Atlas Program (HuBMAP) Method Development Community

### ABSTRACT

A CODEX® Reporter is a fluorescent dye conjugated to an oligonucleotide that is complementary to a specific oligonucleotide barcoded antibody. This protocol describes how to prepare 96-well plates of Reporter Mix for use in a CODEX® run. Each well contains Reporters are grouped together in mixtures of three per cycle plus the nuclear stain. During each cycle the Codex instrument dispenses one well on the tissue sample and images the 3 markers and the nuclear stain. At the end of the cycle the Reporters are removed from the tissue by a gentle wash.

### THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Akoya BiosciencesInc,CODEX User Manual REV A.0 2019

### ATTACHMENTS

[CODEX Cycle Template.xlsx](#)

### GUIDELINES

Plan the CODEX experiment carefully to ensure that each CODEX/barcoded antibody has a unique barcode not shared with any other antibody in the entire multicycle experiment. Assign each antibody a cycle number ensuring that there is only one reporter labeled with a given fluorescence/dye in each cycle.

Each cycle can have one, two or three reporters.

Blank cycles, (no reporter) containing nuclear stain only, should be included at the beginning and end of the experiment. (i.e. cycle 1 and last cycle)

Use the attached cycle template to plan the experiment and enter information into the CODEX run software.

Use only the CODEX supplied 96-well Plates and Foil seals. Other seals may stick to the instrument during sample loading steps.

### MATERIALS TEXT

#### CODEX® KIT

96-well Plates Akoya CODEX 232527

96-well Plate Foil Seals Akoya CODEX 232528

10X CODEX Buffer Akoya CODEX 232119

Assay Reagent Akoya CODEX232120

Nuclear Stain AkoyaCODEX 232121

Reporters (appropriate for barcodes antibodies)

#### Other Materials

Nuclease-Free Water (not DEPC Treated) Ambion AM9938

Amber colored 1.5 ml tubes USA Scientific Sealrite 1615-5507

Ice bucket

# BEFORE STARTING

Thaw Assay reagent, Reporters, and Nuclear Stain and place on ice.

Label a 1.5ml amber microcentrifuge tube for each cycle of the CODEX experiment. Include a Blank cycle at the beginning and end of the experiment.

- 1 Prepare the **Reporter Stock Solution** (Based on the number of cycles and coverslips to be run ):

Prepare Reporter Stock Solution									
Number of cycles (wells)	5	9	10	15	18	20	27	36	45
									cycles (wells)
<b>Nuclease Free Water</b>	1220	2196	2440	3660	4392	4880	6588	8784	10980
<b>10X CODEX buffer</b>	150	270	300	450	540	600	810	1080	1350
<b>Assay Reagent</b>	125	225	250	375	450	500	675	900	1125
<b>Nuclear Stain</b>	5	9	10	15	18	20	27	36	45
<b>Total</b>	1500	2700	3000	4500	5400	6000	8100	10800	13500
Example: # Coverslips@ 9 cycles		1 coverslip			2 coverslips		3 coverslips	4 coverslips	5 coverslips

Amount of Reporter Stock Solution by Number of Cycles Volumes are in ul.

- 2 Prepare the Reporter Master Mix Aliquots for Each Cycle

- 2.1 Label an amber microcentrifuge tube for each cycle. Include tubes for the blank cycles at the beginning and end of the experiment.

- 2.2 Aliquot Reporter Stock Solution into each tube following the chart below (volumes will vary depending on the number of reporters used in an individual cycle (see CODEX Cycle Template)).

<b>Reporter Solutions:</b> <b>Label a 1.5 ml amber tube for each cycle</b>				
	<b>3 Reporters</b>	<b>2 Reporters</b>	<b>1 Reporter</b>	<b>(Blank) 0 Reporters</b>
<b>1 Plate</b> <b>(will add 5ul each reporter)</b>	235 ul	240	245	250
<b>2 Plates</b> <b>(will add 10 ul each reporter)</b>	470 ul	480	490	500
<b>3 Plates</b> <b>(will add 15 ul each reporter)</b>	705 ul	720	735	750
<b>4 Plates</b> <b>(will add 20 ul each reporter)</b>	940 ul	960	980	1000
<b>5 Plates</b> <b>(will add 25 ul each reporter)</b>	1175ul	1200	1225	1250

Reporter Stock Solution volumes by Number of Plates and Number of Reporters per Cycle

- 3 Add appropriate Reporters to the Reporter Stock Solution aliquots to make a Master Mix for each cycle
- 3.1 Place reporters on ice.
- 3.2 *Briefly*, Spin tubes using a bench top centrifuge. Return Reporters to ice.

- 3.3 Pipette Akoya Reporters into the labeled amber tubes containing Reporter Stock Solution. Use volumes listed in step 2.2. column A based on number of reporters in a particular cycle.  
Follow the cycle template or the reporter tray template (below).

<b>CODEX Cycle Template</b>				
	<b>Nuclear Stain</b>	<b>AF 750</b>	<b>550</b>	<b>Cy5</b>
<b>cycle 1 Blank cycle</b>	in Reporter Stock	No Reporter	No reporter	No Reporter
<b>cycle 2 (example: 3 antibodies,-750, 550, and Cy5)</b>	in Reporter Stock	Reporter 1 (matches antibody1)	Reporter 2 ( matches antibody2)	Reporter 3 ( matches antibody3)
<b>cycle 3 (example 2 antibodies- 550 and Cy5)</b>	in Reporter Stock	No Antibody in this wavelength	Reporter 4 ( matches antibody4)	Reporter 5 ( matches antibody5)
<b>cycle 4 (example 1 antibody- 550)</b>	in Reporter Stock	No Antibody in this wavelength	Reporter 6 ( matches antibody6)	No Antibody in this wavelength
<b>5</b>				
<b>6</b>				
<b>7</b>				
<b>8</b>				
<b>*</b>				
<b>last cycle Blank cycle</b>	in Reporter Stock	No Reporter	No reporter	No Reporter

CODEX Cycle Template.

<b>Reporter Tray Template</b>							
<b>Cycle 1</b>	<b>Cycle 2</b>	<b>Cycle 3</b>	<b>Cycle 4</b>	<b>Cycle 5+</b>	<b>Cycle *</b>	<b>Cycle**1</b>	<b>dye/fluor</b>
Blank	Antibody 1 (Reporter 1)	Antibody 4 (Reporter 4)	label any EMPTY channels			Blank	Cy7
	Antibody 2 (Reporter 2)	Antibody 5 (Reporter 5)	Antibody 7 (Reporter 7)				550
	Antibody 3 (Reporter 3)	Antibody 6 (Reporter 6)	...etc				Cy5

Reporter 96-well Plate Template

4 Prepare the 96-well Reporter Plate:

Pipette **245  $\mu$ l** of Reporter containing Master Mix into the corresponding cycle well .



Filled 96-well Reporter Plate with foil seal strips in place

5



Use a different 96-well plate for each Reporter plate prepared. Lightly cover entire rows of wells (i.e. B1-B12) with strips of foil seal.

Keep the filled 96-well plates **IN THE DARK** to avoid photo bleaching.

Store at 4°C for up to two weeks.

 **4 °C**



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