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CODEX Sample Preparation and Staining Experiment Protocol

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CODEX system is the combination of an (1) oligo-nucleotide based antibody labeling-detection technique, (2) a microfluidics instrument coupled with an inverted microscope capable of whole slide scanning, and an (3) ImageJ-based analysis platform. This experimental setup allows the automation of immunofluorescence signal detection across several staining cycles of a single tissue section. This protocol describes detailed methods used in CODEX sample preparation and staining at the Laszik Lab.

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QA/QC Parameters

1. Visual inspection of whole slide digital images of the stains. Every stain (channel-target) in each section is visually evaluated.
2. Positive controls. A positive control stain is performed with each CODEX stain on a "control" kidney with inflammation.
3. Automation. Using standardized operator-independent automated validated protocols.

CODEX data quality management processing

All CODEX data generated go through a rigorous quality control process that includes visual assessment of the processed images and a review of the segmented files, both test specimens and on slide controls. In addition to the stains, cellular and compartmental segmentation fidelity is key to downstream analysis. Unexpected staining pattern and/or artifacts on the processed images are documented; if correctable, such as those related to folding or edge artifact among others, the images are manually annotated to define regions that could be rescued for analytics. If corrective measures are not an option, such as that with a failed stain the data for that particular stain will be excluded from the analysis. Segmented images, both with cellular and compartmental segmentation are also reviewed for accuracy; once again, if abnormal/unexpected segmentation is detected, depending on the nature of such finding either corrective measures are implemented or the case/stain is flagged and excluded from further analysis.

CODEX staining

Antibodies, barcodes, reporters

- CODEX barcoded antibodies
- CODEX barcodes
- CODEX reporters

CODEX Staining Kit

- Staining Buffer
- Storage Buffer
- N Blocker
- G Blocker
- J Blocker
- S Blocker
- Fixative reagent

Consumables, solvents, equipment, glassware, biologics, reagents, instrumentation

- 10X CODEX® Buffer
- CODEX® Gaskets
- Coverslips
- Tissue Processing
- CODEX® Run 96 well plates
- 96 well plate seals
- Assay Reagent
- Nuclear Stain
- Glass beaker (0.5 L) 6 X Glass beakers (50 mL)
- Buffer reservoir
- Buffer tray - Required. No Substitutions. VWR
- Saran wrap
- Plastic petri dish
- Coverslip Prep Cardboard freezer box
- Lab tape
- Bent-tip tweezers
- 6-Well TC Plates - Does not need to be tissue cultured treated.
- 1mL, 1.5 mL, 2 mL
- Amber 1.5 mL tubes
- 5, 15, 50 ml conical tubes
- 16% Paraformaldehyde Staining Tissue
- 1X PBS
- Conjugation,
- Poly-L-Lysine 0.1%
- Drierite Absorbents
- Nuclease-Free Water
- Fluoromount-G™ (optional)
- MilliQ H2O
- Acetone
- Methanol
- DMSO - ACS reagent, ≥99.9%
- CODEX instrument
- CODEX software suite

CODEX Conjugation

CODEX Conjugation Kit

- Filter Blocking Solution
- Reduction Solution 1
- Reduction Solution 2
- Conjugation Solution
- Purification Solution
- Antibody Storage Solution

Consumables, solvents, equipment, glassware, biologics, reagents, instrumentation

- 50kDa MWCO filter
- Screw-top 1 or 2mL tubes
- Customer choice
- Parafilm
- NuPAGE™ LDS Sample Buffer (14X)
- NuPAGE™ Sample Reducing Agent (10X)
- Conjugation QC
- NuPAGE™ 4-12% Bis-Tris Protein Gels Thermo Fisher Scientific NP0321BOX Conjugation QC
- Novex™ Sharp Pre-Stained Protein Standard 3.5-260 kDa
- Novex™ SimplyBlue™ SafeStain NuPAGE™ MOPS SDS Running Buffer (20X)
- XCell SureLock™ Mini-Cell Electrophoresis System
- 95°C dry bath
- Nanodrop
- Shaker
- Microwave

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Tissue Sample Preparation Prior to CODEX Staining

12h 1m

1 Gather necessary materials





Materials needed:

- Coverslips (CODEX Mounting Kit 1.0 @RT)

- Poly-Lysine Solution 1% (CODEX Mounting Kit 1.0 @RT)
- 600 mL glass beaker
- Rubber band
- Saran wrap
- Milli-Q water
- Plastic petri dish
- Paper towels

2 Coat coverslips with poly-lysine.

Incubating the coverslips in poly-lysine will need to take place least 12 hours prior to embedding tissue samples

- 2.1 Gently place 20 coverslips on the bottom of the glass beaker and slowly swirl the beaker to fan out the coverslips.
- 2.2 Add  **70 mL** of poly-lysine solution to the beaker, making sure to submerge all coverslips.
- 2.3 To prevent evaporation, use the saran wrap to cover the beaker and seal the wrap with a rubber band.
- 2.4 **STOPPING POINT-** Incubate coverslips in poly-lysine solution for a minimum^{12h} of  **12:00:00** and up to one week at  **Room temperature** .
- 2.5 After incubation, dispose of the poly-lysine solution into a proper waste container.
- 2.6 Fill the beaker halfway with Milli-Q water and swirl contents to rinse coverslips.
- 2.7 Let the beaker and coverslips sit for  **00:01:00** .

1m

2.8 Dispose of the water into the sink.

2.9 Repeat steps 2.5-2.8 for a total of 7 washes.

2.10 Fill the beaker halfway with Milli-Q water.

2.11 Place two sets of paper towels on the benchtop.

2.12 Remove the coverslips from the beaker, then separate and lay each piece face down on the first set of paper towels.

2.13 Invert each coverslip to dry the reverse side on the second set of paper towels.

2.14 Once the coverslips dry completely, collect them in a plastic petri dish to store for up to 2 months.

3 Cut and embed frozen tissues on poly-lysine coated coverslips.

3.1 Place poly-lysine coated coverslips in the cryostat chamber to equilibrate.

3.2 Cut frozen tissue slices with \rightarrow **5 μ m** - \rightarrow **10 μ m** thickness.

Do not exceed \rightarrow **10 μ m**

DO NOT EXCEED 10 min

- 3.3 Gently place tissue slice in the center of the coverslip.
- 3.4 Place a gloved finger on the underside of the coverslip behind the tissue slice to melt the OCT for better adherence. Do not exceed 2 seconds.
- 3.5 Store tissue slices separated at **-80 °C** for up to 6 months prior to staining.

Antibody Conjugation with CODEX-tags for Custom Panel





5h 14m 5s



4 Gather necessary materials.




Material needed:

- Antibody Reduction Solution 2 (CODEX Conjugation Kit 1.0 @ 4C)
- Antibody Conjugation Solution (CODEX Conjugation Kit 1.0 @ 4C)
- Filter Blocking Solution (CODEX Conjugation Kit 1.0 @ 4C)
- Antibody Reduction Solution 1 (CODEX Conjugation Kit 1.0 @-20C, thaw right before use, one tube for every 3 antibodies)
- CODEX Antibody Tags (CODEX Conjugation Kit 1.0 @-20C, thaw right before use)
- Antibody Purification Solution (CODEX Conjugation Kit 1.0 @ 4C)
- Antibody Storage Solution (CODEX Conjugation Kit 1.0 @ 4C)
- Purified Antibodies (50 µg needed per antibody)
- 50 kDa MWCO filter
- 2 mL screw-top tubes
- Milli-Q water

- 5 Calculate and obtain **50 µg** of each antibody that will be conjugated.
- 6 Label a 50kDa MWCO filter unit (both the filter device and the collection tube) for each antibody that will be conjugated.




- 7 Add  **500 μ L** of Filter Block Solution to the filter device.
- 8 Spin down the filter unit at  **12000 x g, 00:02:00** . 2m
- 9 Discard all liquid (from both the filter device and the collection tube).
- 10 Add  **50 μ g** of antibody to the filter device.
- 11 Spin down the filter unit at  **12000 x g, 00:08:00** . 8m
- 12 Prepare the Antibody Reduction Master Mix while spinning.
- For one antibody, mix the following:

 **6.6 μ L** Antibody Reduction Solution 1 +  **275 μ L** Antibody Reduction Solution 2

Multiply the solution 1 and solution 2 volumes by the total number of antibodies that will be conjugated to get the total volume needed from each reagent.
- 13 Discard flow through.
- 14 Add  **260 μ L** of Antibody Reduction Master Mix to each filter device.
- 15 Vortex solution in filter device for  **00:00:02** -  **00:00:03** . 5s

- 16 Incubate tube for 🕒 **00:30:00** at 🌡 **Room temperature** . 30m
- 17 Spin down the filter unit at 🌀 **12000 x g, 00:08:00** . 8m
- 18 Discard flow-through.
- 19 Add 📏 **450 µL** of Antibody Conjugation Solution to each filter device.
- 20 Spin down the filter unit at 🌀 **12000 x g, 00:08:00** . 8m
- 21 Prepare the CODEX antibody tag solution while spinning.
- 21.1 Label a new tube for each CODEX antibody tag (one tag per antibody).
- 21.2 Add 📏 **210 µL** of Antibody Conjugation Solution to each tube in step a. Set aside.
- 21.3 Add 📏 **10 µL** of Milli-Q water to resuspend each CODEX antibody tag (retrieved from -20 just prior). Mix by pipetting.
- 21.4 Add the entire resuspended CODEX antibody tag to step b and mix by pipetting.

- 22 Discard flow-through.
- 23 Add the CODEX antibody tag solution prepared in step 21 to the corresponding filter device.
- 24 Close the lid and vortex the filter unit for 2-3 sec.
- 25 Incubate tube for 🕒 **02:00:00** at 🌡 **Room temperature** . 2h
- 26 After 🕒 **02:00:00** , set aside 🧴 **5 µL** of the purified solution for QC. 2h
- 27 Spin down the filter unit at 🌀 **12000 x g, 00:08:00** . 8m
- 28 Discard flow-through.
- 29 Add 🧴 **450 µL** of Antibody Purification Solution to each filter device.
- 30 Spin down the filter unit at 🌀 **12000 x g, 00:08:00** . 8m
- 31 Discard flow-through.
- 32 Repeat steps 29-31 two more times for a total of three times.

- 33 There will be solution left in the filter device.
- 34 Label a new collection tube for each filter device.
- 35 Add  **100 µL** Antibody Storage Solution to each filter device.
- 36 Invert the filter device into its newly labeled collection tube.
- 37 Spin down the filter unit at  **3000 x g, 00:02:00** . 2m
- 38 Transfer solution in the collection tube to a labeled 2 mL screw-top tube and store at  **4 °C** for up to 1 year.

Tissue Staining 5h 14m 5s

- 39 Gather necessary materials.

Materials needed:

- Hydration Buffer (CODEX Staining Kit 1.0 @ 4C)
- Staining Buffer (CODEX Staining Kit 1.0 @ 4C)
- Storage Buffer (CODEX Staining Kit 1.0 @ 4C)
- Blocking Component N (CODEX Staining Kit 1.0 @ 4C)
- Blocking Component J (CODEX Staining Kit 1.0 @ 4C)
- Blocking Component G (CODEX Staining Kit 1.0 @ -20C)
- Blocking Component S (CODEX Staining Kit 1.0 @ -20C)
- CODEX-Tagged Antibodies (CODEX Antibody Kit 1.0 @ -20C)
- Fixative (F) Aliquots (CODEX Staining Kit 1.0 @ -20C, thaw right before use, one tube for every 5 tissue samples)
- Acetone
- Methanol (Keep in -20C prior to starting experiment)

- 16% PFA
- 1 x DPBS
- 6-well TC plates
- Bent-tip tweezers
- Drierite Absorbents
- Eppendorf tubes
- Conical tubes
- 50 mL beakers
- Empty pipet tip boxes

40 Prepare a humidity chamber.

40.1 Place wet paper towels at the bottom of an empty pipet tip box.

40.2 Fill the box with enough water to cover the towels completely.

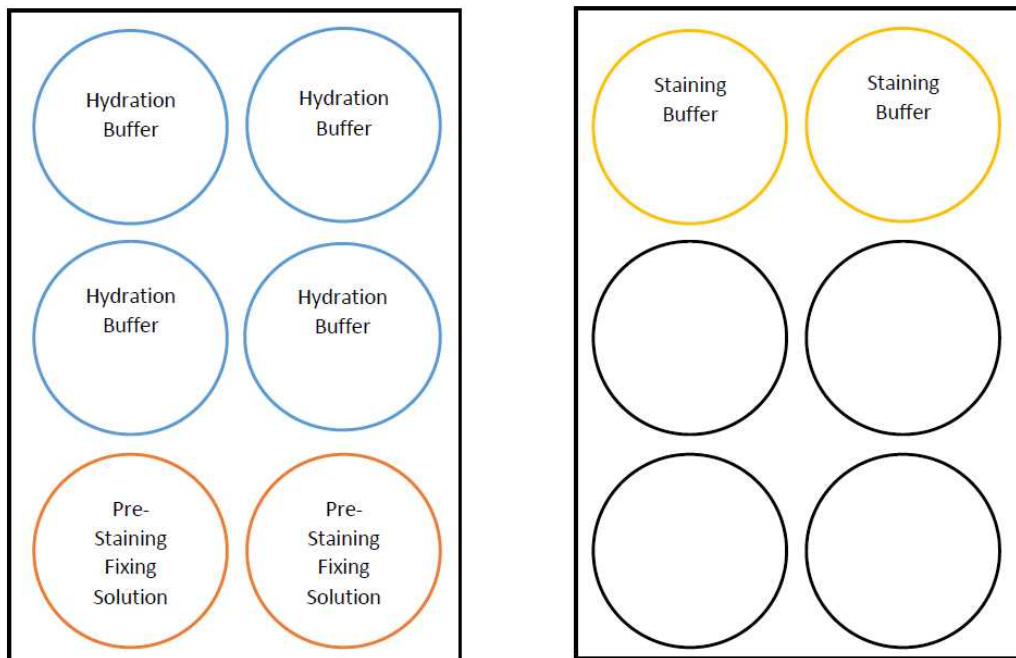
40.3 Recover the pipet tip tray and lid after rinsing.

41 Prepare Drierite absorbent beads.

41.1 Fill an empty pipet tip box with Drierite beads (about 1~2 cm deep).

42 Prepare the following plate configuration for CODEX solutions:

For two tissue samples:



*5 mL of each solution per well



43 Prepare of acetone in a beaker for each tissue.

44 Remove the tissue sections from -80C and place the coverslips on Drierite absorbent beads.

45 Let the tissue sections dry for at . 2m

46 Place the tissue samples face-up in the beakers containing acetone.

47 Incubate for . 10m

48 Remove the coverslips from acetone and place them in the humidity chamber to dry for  00:02:00 at  Room temperature . 2m

49 Hydrate tissue samples following the plate configuration.

49.1 Place the coverslips in the Hydration Buffer wells (dip 2-3 times before fully submerging the slide). Ensure tissue is facing up.

49.2 Incubate for  00:02:00 . 2m

49.3 Place the coverslips in the next set of Hydration Buffer wells.

49.4 Incubate for  00:02:00 . 2m

49.5 Prepare Pre-Staining Fixing Solution while incubating tissue.

For two samples, mix the following:

 1 mL 16% PFA +  9 mL Hydration Buffer

Multiply the PFA and buffer volumes by the total number of samples that will be stained to get the total volume needed from each reagent. Add

 5 mL of Fixing Solution 1 to each well.

49.6 Place the coverslips in the Pre-Staining Fixing Solution wells.

49.7 Incubate for  00:10:00 at  Room temperature . 10m

49.8 Remove samples from Pre-Staining Fixing Solution and rinse the samples by dunking the coverslips 2-3 times each in the top and bottom Hydration Buffer wells.

49.9 Place the samples in the Staining Buffer wells and let equilibrate for 50m
🕒 00:20:00 - 🕒 00:30:00 .

50 Prepare the antibody cocktail during equilibration.

50.1 To make CODEX Blocking Buffer for two samples, mix the following:

🧴 10 µL Blocking Component N + 🧴 10 µL Blocking Component G +
🧴 10 µL Blocking Component J + 🧴 10 µL Blocking Component S +
🧴 380 µL Staining Buffer

Multiply the reagent volumes by the total number of samples that will be stained to get the total volume needed for the experiment.

50.2 Calculate the total antibody volume per antibody cocktail.

Total Ab volume = (# of antibodies) x (# of samples) x (Ab volume/sample)]

E.g. To stain two tissue samples with 24 antibodies and assuming 1 µL of antibody is needed per sample:

Total Ab volume = 24 antibodies x 2 samples x 1 µL/sample = 48 µL

50.3 Calculate to CODEX Blocking Buffer needed per antibody cocktail.
CODEX Blocking Buffer volume needed to make 210 µL Ab Cocktail
Solution/sample = CODEX Blocking Buffer volume - Total Ab volume

E.g. For two tissue samples with a total Ab volume of 48 µL:

CODEX Blocking Buffer volume needed for Ab Cocktail Solution = 420 µL

Blocking Buffer volume - 48 µL total Ab volume = 372 µL

-> Ab Cocktail Solution = 48 µL Ab volume + 372 µL CODEX Blocking

50.4 Combine the antibodies and the CODEX Blocking Buffer to make **210 µL** Antibody Cocktail Solution/tissue sample.

51 Remove the coverslips from Staining Buffer and place in the humidity chamber.

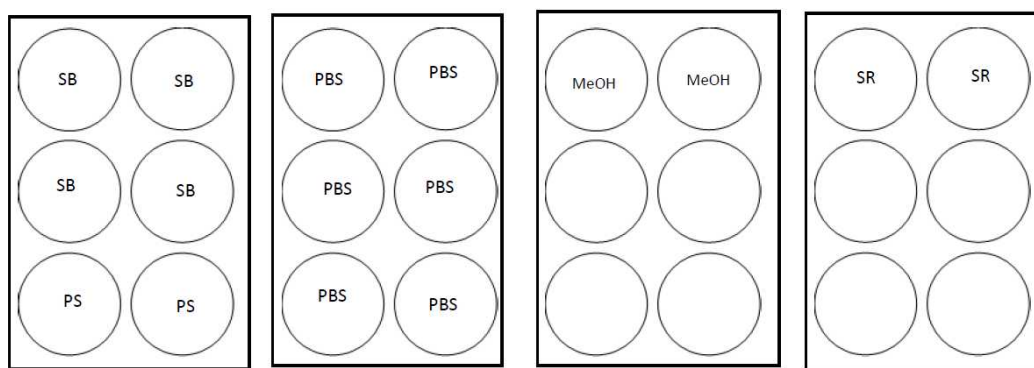
52 Quickly add **200 µL** of Antibody Cocktail Solution to each sample from the top corner of the coverslip.

53 Place lid back on the humidity chamber.

54 Incubate for **03:00:00** at **Room temperature**.

3h

55 Prepare the following plate configuration for CODEX solutions ~1 hour before the 3-hour incubation ends:



*5 mL of each solution per well. Schematic is for two tissue samples.

56 Wash the tissue samples following the plate configuration.

- 56.1 Place the coverslips in the Staining Buffer (**SB**) wells (dip 2-3 times before fully submerging the slide). Ensure tissue is facing up.
- 56.2 Incubate for 🕒 **00:02:00** . 2m
- 56.3 Place the coverslips in the next set of Staining Buffer (**SB**) wells.
- 56.4 Incubate for 🕒 **00:02:00** . 2m
- 56.5 Prepare Post-Staining Fixing Solution (PS) while incubating tissue.
For two samples, mix the following:
- 🧴 **1 mL 16% PFA** + 🧴 **9 mL Storage Buffer**
- Multiply the PFA and buffer volumes by the total number of samples that will be stained to get the total volume needed from each reagent. Add 🧴 **5 mL** of Post-Staining Fixing Solution to each well.
- 56.6 Place the coverslips in the Post-Staining Fixing Solution (PS) wells.
- 56.7 Incubate for 🕒 **00:10:00** at 🌡 **Room temperature** . 10m
- 56.8 Prepare ice-cold methanol during incubation.
- Place a 6-well TC plate on ice.
 - Pipet methanol up and down to equilibrate the serological pipet tip.
 - Add 5 mL methanol to one well per sample.
- 56.9 Remove samples from Post-Staining Fixing Solution (**PS**) and rinse the samples by dunking the coverslips 2-3 times each in the 1x DPBS (**PBS**) wells

for a total of 3 washes.

56.10 Remove the coverslips from 1x DPBS (**PBS**) and place in ice-cold methanol.

56.11 Incubate for 🕒00:05:00 . 5m

56.12 Bring plate containing 1x DPBS (**PBS**) to the ice bucket for quick transfer from methanol.

56.13 After incubation, rinse the samples by dunking the coverslips 2-3 times each in the 1x DPBS (**PBS**) wells for a total of 3 washes.

57 Rinse the humidity chamber if not previously washed.

58 Prepare the Final Fixative Solution. For 1-5 samples, mix the following:

🧴1000 µL 1x DPBS + 🧴20 µL CODEX Fixative Reagent

*20 µL of CODEX Fixative Reagent is one aliquot, thaw and spin down right before use.
Vortex gently to mix.


59 Remove coverslips from wells and place them in the humidity chamber.

60 Add 🌡️ Room temperature of Final Fixative Solution to the top corner of each sample.

61 Incubate for 🕒00:20:00 . 20m

62 Remove samples from the humidity chamber and rinse the samples by dunking the coverslips

2-3 times each in the 1x DPBS (**PBS**) wells for a total of 3 washes.

- 63 Place the coverslips in a new TC plate with  **5 mL** Storage Buffer (SB) per sample in each well.
- 64 **STOPPING POINT**- use samples directly to run a CODEX experiment or store up to two weeks at 4C.

CODEX-tagged Dyes Cycle Prep





- 65 Gather necessary materials:

Materials needed:

- 96-well plates (CODEX Mounting Kit 1.0 @ RT)
- 96-well plate foil seal (CODEX Mounting Kit 1.0 @ RT)
- 10X CODEX Buffer (CODEX Assay Kit 1.0 @ RT)
- CODEX Assay Reagent (CODEX Assay Kit 1.0 @ -20C)
- CODEX Nuclear Stain (CODEX Assay Kit 1.0 @ -20C)
- CODEX-Tagged Dyes (CODEX Antibody Kit 1.0 and CODEX Conjugation Kit 1.0 @ -20C)
- Nuclease-free water
- 1.5 mL amber Eppendorf tubes

- 66 Determine the CODEX-tagged dyes combination for each running cycle. Each cycle may contain up to one HOECHST/DAPI dye, one FAM/FITC dye, one Cy3/TRITC dye, and one Cy5 dye.
- 67 Label an amber Eppendorf tube for each CODEX-tagged dyes combination.
- 68 Prepare the stock solution of CODEX Reporter Stock Solution.

For 5 wells (cycles) per plate, mix the following:

 **150 µL** 10x CODEX Buffer +  **125 µL** Assay Reagent +  **5 µL** Nuclear Stain
+  **1220 µL** Nuclease-free water

Double or triple the volume of each reagent if running 10 or 15 cycles per plate. Mix solution by gently inverting.

69 Add CODEX Reporter Stock Solution to each labeled amber Eppendorf tube.


| A | B | C | D |
|---|---------------------|---------------------|--------------------|
| | 3 CODEX-tagged dyes | 2 CODEX-tagged dyes | 1 CODEX-tagged dye |
| CODEX Reporter Stock Solution Volume per tube | 235 µL | 240 µL | 245 µL |

70 Thaw each CODEX-tagged dye immediately before use.

71 Quickly spin down the CODEX-tagged dyes.

72 Add  5 µL of each CODEX-tagged dye to its corresponding tube.

73 Mix contents of each tube by gently pipetting.

74 Transfer  245 µL of solution from each tube to its designated well in the 96-well plate.

75 Prepare a strip of foil seal and cover all wells containing CODEX-tagged dyes.

76 STOPPING POINT- use dyes directly to run a CODEX experiment or store up to two weeks at 4C.

Running the CODEX instrument 20m



77 Gather necessary materials.

Materials needed:

Materials needed:

- 96-well plate foil seal (CODEX Mounting Kit 1.0 @ RT)
- 2 CODEX Gaskets (CODEX Mounting Kit 1.0 @ RT)
- 10X CODEX Buffer (CODEX Assay Kit 1.0 @ RT)
- CODEX Nuclear Stain (CODEX Assay Kit 1.0 @ -20C)
- Stage Insert with Lid (CODEX Instrument)
- 600-2000 mL glass beakers
- Bent-tip tweezers
- 1.5 mL and 2 mL Eppendorf tubes
- Milli-Q water
- DMSO
- Aspirator setup
- Pipette tip box lid
- Ice Bucket



78 Turn on the CODEX Instrument.

79 Remove the antibody-stained tissue section(s) adhered to the Poly-lysine coated coverslip^{15m} and the sealed, pre-loaded 96-well plate containing the Reporter Master Mix solutions from 4C. Allow them to equilibrate to  **Room temperature** for at least  **00:15:00**.

80 Prepare 1X CODEX Assay Buffers in 600-2000 mL glass beakers.

| A | B | C | D | E | F | G | H | I |
|------------------|----------------|----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| | 5 CODEX cycles | 8 CODEX cycles | 10 CODEX cycles | 12 CODEX cycles | 15 CODEX cycles | 18 CODEX cycles | 20 CODEX cycles | 25 CODEX cycles |
| 10X CODEX Buffer | 31 mL | 44 mL | 53.3 mL | 63.3 mL | 78.5 mL | 93.5 mL | 103.5 mL | 128.5 mL |
| Milli-Q water | 281 mL | 394 mL | 480.5 mL | 570.5 mL | 705.5 mL | 840.5 mL | 930.5 mL | 1155.5 mL |
| Total | 312 mL | 438 mL | 534 mL | 634 mL | 784 mL | 934 mL | 1034 mL | 1284 mL |
| DMSO | 236 mL | 318 mL | 386 mL | 454 mL | 556 mL | 658 mL | 726 mL | 896 mL |

1X CODEX Buffer

Set aside  **7 mL** of 1x CODEX Buffer in a  **15 mL** conical tube for later use.

81 Place generated 1x CODEX Buffer in the CODEX Buffer Bottle, labeled Bottle One and DMSO in

the amber bottle, labeled Bottle Two.

82 Load the stage insert.

- 82.1 Untwist the knobs at the top of the stage insert to remove the lid with the fluidic lines.
- 82.2 Remove the old coverslip using the forceps, push the corners of the gaskets towards the center to release the seal between the coverslip and the gasket.
- 82.3 Gently remove the coverslip using the forceps.
- 82.4 If the coverslip breaks, remove all glass pieces and rinse with water.
- 82.5 Soak fresh gaskets in 1X CODEX Buffer or in Storage Buffer for a few seconds.
- 82.6 Place the first gasket inside the squared well at the center of the stage insert.
- 82.7 Gently tap with forceps to ensure it adheres to the stage insert surface.
- 82.8 Gently place the tissue sample on top of the gasket, making sure it is perfectly inserted and the tissue is facing upwards.
- 82.9 Quickly and gently tap the coverslip edges with the forceps to ensure it adheres to the gasket.

82.10 Quickly place the second gasket on top of the sample coverslip.

82.11 Quickly place the lid on top of the stage insert.

82.12 Secure the lid by turning the knobs.

82.13 Dispense  700 μL of 1X CODEX Buffer on the corner of the sample.

82.14 Remove any salts formed on the coverslip from drying using a kimwipe with Milli-Q water. Use a dry kimwipe to remove residual liquid.

83 Stain the CODEX sample with HOECHST.

83.1 Thaw CODEX Nuclear Staining Solution.

83.2 Have the  7 mL of 1x CODEX Buffer set aside previously within reach.

83.3 Prepare a  2 mL Eppendorf tube for the Nuclear Stain Solution.

Prepare the Nuclear Stain Solution.

 1999 μL 1x CODEX Buffer +  1 μL Nuclear Staining Solution

83.4 Remove the solution within the sample well.

83.5 Add  700 μL of Nuclear Stain Solution to the sample.

83.6 Incubate for  00:05:00 at  Room temperature . Cover it from light. ^{5m}

83.7 Remove the solution within the sample well.

83.8 Add 700 μL of 1x CODEX Buffer.

83.9 Repeat steps 83.7-83.8 for a total of 5 washes.

83.10 Add  700 μL of 1x CODEX Buffer.

84 Launch the CODEX Instrument Management Software and enter cycle, marker id and exposure time information. Minimize the window.

85 Place the plate with the CODEX-tagged dyes in its designated place in the instrument.

86 Clean any dried buffer on the bottom of the coverslip with a wet kimwipe and dry off any residual liquid.

87 Turn on the Keyence scanner and load the cassette into the scanner.

