May 16, 2019

Working

Fresh frozen tissue staining with CODEX tagged antibody panel

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dx.doi.org/10.17504/protocols.io.2qpgdvn

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ABSTRACT

CODEX is a technology that uses oligo labeled antibodies, specialized fluorescent probes, and a companion instrument along-side a standard fluorescence microscope to create single-cell resolution fluorescence data across a multitude of parameters within spatial context in a single tissue. CODEX was developed by Yury Goltsev and Nikolay Samusik in the laboratory of Garry Nolan at Stanford. The technology is being commercialized by Akoya Biosciences.

EXTERNAL LINK

https://www.cell.com/cell/pdf/S0092-8674(18)30904-8.pdf

GUIDELINES

Ensure that tissues never dry out after the rehydration step.

Never pipet directly on top of tissue. Dispense liquids on the corner of the coverslip.

Coverslips are best handled with the bent-tip tweezers found in the materials section.

MATERIALS TEXT

PRODUCT	PROVIDER	CATALOG NUMBER
Coverslip Staining Jar	Ted Pella	21040
Bent tip forceps	Fine Science Tools	11251-33
6-well TC plates	VWR	10861-554
16% PFA	EMS	15710
1X DPBS	Thermofisher	14190144
Drierite	Fisher Scientific	23-116582
Acetone	Sigma-Aldrich	650501
Methanol	Sigma-Aldrich	34860
Staining Buffer 1	Akoya	RGT2001EA
Staining Buffer 2	Akoya	RGT2001EA
Staining Buffer 3	Akoya	RGT2001EA
Blocking Component 1	Akoya	RGT2001EA
Blocking Component 2	Akoya	RGT2001EA
Blocking Component 3	Akoya	RGT2001EA
Blocking Component 4	Akoya	RGT2001EA
Fixative (F) aliquots	Akoya	RGT2001EA
CODEX tagged antibodies	Akoya	RGT2000EA

BEFORE STARTING

Antibody staining volumes should be determined by prior titration experiments. A good start is 1ul per 200ul cocktail.

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Create a humidity chamber by re-purposing a pipet tip box. Remove the tray with holes that holds the tips, place a wet paper towel in the bottom of the box, replace tray, and cover with lid.

Tissue Preparation

1 Begin with fresh frozen tissues sectioned onto 22mm^2 poly-lysine coated coverslips and stored at -80C.

Add a 5mmlayer of drierite granules on the bottom of a pipet tip box or similar container. Place a piece of kimwipe on top of the drierite. Retrieve the coverslips from freezer with bent-tip tweezers and place on top of the kimwipe, tissue-side-up.

A scratch with the tip of the tweezers on the OCT layer can determine which side of the coverslip has the tissue.

Close the box and let sit at RT for 2 minutes.

2 Transfer coverslips to a coverslip staining jar containing acetone.

Incubate at RT for 10 minutes.

3 Arrange coverslips on a paper towel, tissue-side-up, and allow to air-dry for 2 minutes.

4 Fill the wells of a 6-well TC plate with ~5ml ***Staining Buffer 1*** and transfer the coverslips into the wells for the first rehydration step.

Incubate for 2 minutes.

5 Fill the wells of another 6-well TC plate with ~5ml ***Staining Buffer 1*** and transfer the coverslips into the wells for a second rehydration step.

Incubate for 2 minutes.

The first 6-well TC plate can be rinsed out with ddH_2O and re-used. Several TC plates can be rinsed, re-used, and rotated for the duration of the protocol.

6 Prepare fixing solution in a conical tube by making a 10-fold dilution of 1.6% PFA in ***Staining Buffer 1.*** Each coverslip will require 2ml of fixing solution.

7 Add 2ml fixing solution to new wells and transfer the coverslips into the fixing solution.

Incubate for 10 minutes.

8 After the fixation, transfer the coverslips to wells containing ~5ml ***Staining Buffer 1***.

9 Transfer the coverslips to new wells containing ~5ml ***Staining Buffer 1.***

10 Transfer the coverslips to new wells containing ~5ml ***Staining Buffer 2,*** and allow to equilibrate for at least 2 minutes. Tissues can stay in this solution for up to 30 minutes while antibody cocktail is being prepared.

Antibody Cocktail Preparation

11 The antibody cocktail is a combination of all the antibodies in the panel plus ***CODEX Blocking Buffer.***

Prepare 200ul ***CODEX Blocking Buffer*** for each coverslip to be stained.

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180ul ***Staining Buffer 2***
5ul ***Blocking component 1***
5ul ***Blocking component 2***
5ul ***Blocking component 3***
5ul ***Blocking component 4***

200ul total for each coverslip

12 The antibody volume needed per coverslip should have been determined by prior titration experiments.

A good starting volume is 1ul per coverslip in a 200ul cocktail.

Determine the total antibody volume that will go into the cocktail. The antibody cocktail is the entire antibody panel volume plus a volume of ***CODEX Blocking Buffer*** to equal 200ul.

example: 27ul antibodies + 173ul ***CODEX Blocking Buffer*** = 200ul antibody cocktail
18ul antibodies + 182ul ***CODEX Blocking Buffer*** = 200ul antibody cocktail

13 Combine antibodies and ***CODEX Blocking Buffer*** and gently mix with pipet.

Staining

14 Remove coverslips from ***Staining Buffer 2***, dab the corner on a kimwipe to remove most of the liquid, place tissue-side-up on top of the humidity chamber, and pipet 190ul antibody cocktail on top.

Take care to not pipet directly on top of the tissue. Surface tension should hold the entire volume on top of the coverslip. Do not allow tissues to dry out.

15 Close the humidity chamber and incubate at RT for 3 hours.

Post-processing of Stained Tissue

16 Take coverslips, dab a corner on a kimwipe to remove most of the liquid, and place into wells containing ~5ml ***Staining Solution 2.***

Incubate 2 minutes.

17 Transfer coverslips to new wells with ***Staining Solution 2*** for a total of 2 washes.

Incubate 2 minutes.

18 Prepare fixing solution in a conical tube by making a 10-fold dilution of 1.6% PFA in ***Staining Buffer 3.*** Each coverslip will require 2ml of fixing solution.

19 Add 2ml fixing solution to new wells and transfer the coverslips into the fixing solution.

Incubate for 10 minutes.

20 During the fixation, place a 6-well TC plate on a bed of slushy ice. Allow the plate to chill for 2 minutes and then add ~5ml of ice cold (4C) methanol to one well per coverslip.

21 After the 10 minute fixation, transfer the coverslips to wells containing ~5ml PBS.

Transfer the coverslips to new wells containing PBS

Transfer the coverslips to new wells containing PBS, for a total of 3 washes.

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22 Transfer the coverslips to the ice cold methanol.

Incubate for 5 minutes.

23 After the 5 minute incubation, transfer the coverslips to wells containing ~5ml PBS. Methanol dries fast so this step should be done quickly. Ensure that the coverslip sinks all the way to the bottom of the well.

Transfer the coverslips to new wells containing PBS

Transfer the coverslips to new wells containing PBS, for a total of 3 washes.

24 Prepare 200ul ***Final Fixative Solution*** for each coverslip.

One aliquot of ***CODEX Final Fixative (F)*** yields 1ml ***Final Fixative Solution*** for 5 coverslips.

Cut aliquots from the ***Final Fixative (F)*** strip, allow to thaw, and quickly spin down.

25 Pipet 20ul ***Final Fixative (F)*** into 1ml PBS. Gently mix with pipet.

Final Fixative Solution	**1-5 coverslips**	**6-10 coverslips**
PBS	1000ul	2000ul
(F) Aliquots	20ul	40ul

26 Rinse and dry humidity chamber tray to remove any residual antibodies from the staining. Take the coverslips, dab the corners on a kimwipe, and place them on the humidity chamber.

27 Add 190ul ***Final Fixative Solution*** on top of each coverslip, taking care to not pipet directly on the tissue. Ensure that the entire tissue is covered in solution. Close humidity chamber.

Incubate for 20 minutes.

28 After the 20 minute fixation, transfer the coverslips to wells containing ~5ml PBS.

Transfer the coverslips to new wells containing PBS

Transfer the coverslips to new wells containing PBS, for a total of 3 washes.

29 Store the stained and fixed samples in a 6-well TC plate in ~5ml ***Staining Buffer 3*** at 4C for up to 2 weeks.

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