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Staining Sequenza

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1 Works for me dx.doi.org/10.17504/protocols.io.bmc6k2ze

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ABSTRACT

This protocol describes the use of sequenza staining device. The sequenza can be use for MIBI or IHC staining methods.

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PROTOCOL CITATION

Marc MB Bosse, Sean Bendall, Mike Angelo 2021. Staining Sequenza. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bmc6k2ze

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MATERIALS TEXT

A	B	C
Product	Provider	Cat.Number
Thermo Scientific Shandon Glass Coverplates	Fisher Scientific	72-110-017
Thermo Scientific Shandon Sequenza Immunostaining Center Accessories, slide rack	Fisher Scientific	73-310-017

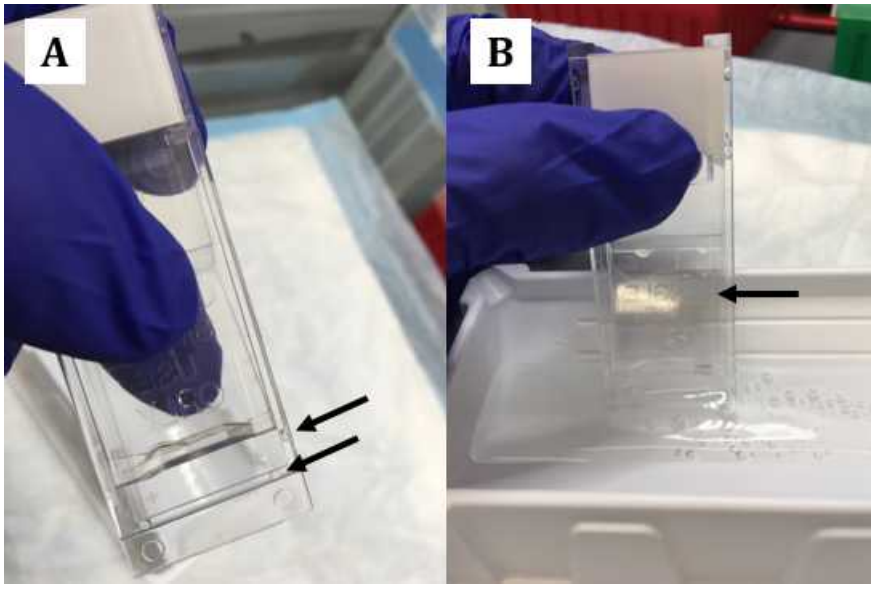
Slide preparation

1 The slide preparation is the same as described in the protocol MIBI staining dx.doi.org/10.17504/protocols.io.bh9zj976

Sequenza assembly

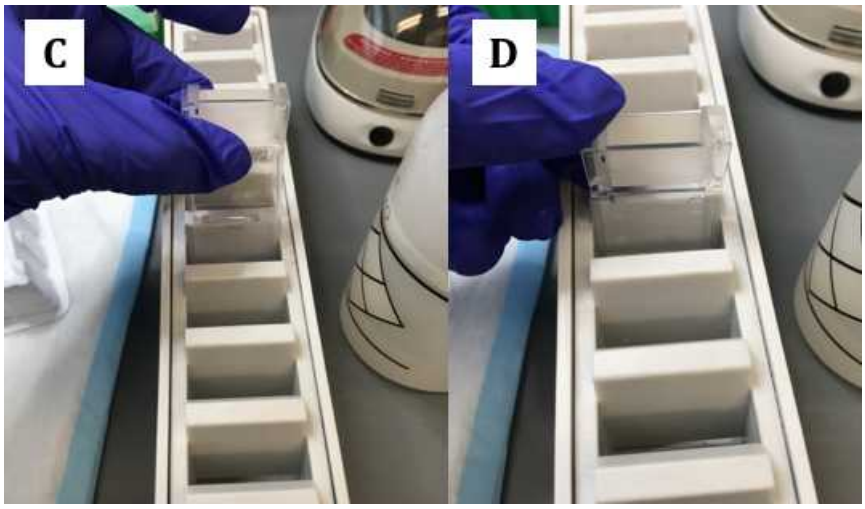
2 Fill a disposable Pipetting Reservoir with **20 mL** of 1x PBS wash buffer

3 Place sample slide on a Sequenza cover plate aligning the bottom slide with the notches on the cover plate (see picture below panel A)



4 Fill by capillarity the space between the slide and cover plate by holding the parts tight and dipping the bottom part of the assembly in the wash buffer reservoir (see picture above panel B)

5 Transfer the slide and coverplate assembly into the Sequenza rack. Slip in the assembly (see below picture panel C)



6 Secure the assembly and make sure that the assembly placed down in the rack (see above picture panel D)

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7 Add **1 mL** of wash buffer. The buffer should flow thru within 1 min 30 s. Repeat by adding 1 mL of wash buffer

Optional: blocking endogeous biotin

8 If you are using biotinylated antibody or probe, you need to block endogenous biotin

Add 3 drops of Avidin solution sufficient to cover the sample and incubate for **00:10:00** **Room temperature**

9 Add 1 mL of wash buffer

10 Add drops Biotin solution sufficient to cover the sample and incubate for **00:10:00** **Room temperature**

11 Add **1 mL** of wash buffer

11.1 If presence of bubbles are observed after adding Avidin and Biotin solution.

Dismount the slide and the Sequenza cover plate

11.2 Fill by capillarity the space between the slide and cover plate by holding the parts tight and dipping the bottom part of

the assembly in the wash buffer reservoir (see picture above panel B)

12 Next day, use anti-biotin metal-labeled antibody (1D4-C5) in ***Stain 2*** panel

Blocking

13 Add **200 µl** of Blocking buffer)

14 Place the cover and incubate with the blocking buffer for 1h

Prepare antibody master mix

15 Spin down briefly all antibody tubes at **10.000 x g** for **00:01:00** , preferably at **4 °C**

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16 The total volume needed for staining each Sequenza slide assembly is **120 µl**

The manufacturer recommend to use 100 µL per Sequenza slide assembly. 120 µL is therefore a 20% excess.

16.1 Prepare a panel table in excel including the following information:

Conjugation ID, Target name, Channel, , Antibody concentration, Titer, Volume

Exemple:

A	B	C	D	E	F
ID	**Target name**	**Channel**	**Concentration (ug/mL)**	**Titer (ug/mL)**	**Calculation formulation for Volume (µL)**
1299	CD4	143	50	1	3
1221	CD11c	161	50	0.25	0.75
1292	CD3	159	50	0.5	1.5
...	...	...	...	...	
				Total volume (µL)	150
				Antibody diluent volume	144.75
				Antibody mix volume	5.25

16.2 Add first, antibody diluent (NHS 3%) to complete to the total volume

17 Pipet the respective amount of each individual antibody. Do not disturb the bottom of the antibody tube when pipetting

18 Prepare 0.1 µm filter unit

18.1 Add 400 µL of antibody diluent buffer (NHS 3%) to a 0.1 µm centrifugal filter device

18.2 Spin at 10,000 x g for 1 min

18.3 Discard the flowthough by aspirating

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18.4 Add the antibody mix to the filter unit

18.5 Spin at 10,000 x g for 1 min

Stain 1 (overnight)

19 After, blocking incubation, add **200 µl** of antibody buffer (NHS 3%)

20 Make sure that the antibody buffer has passed through to prevent dilution of antibody mix. Note: this should take no more than 2 min

21 Add antibody master mix, **120 µl**

The manufacturer recommend to use 100 µL per Sequenza slide assembly. 120 µL is therefore a 20% excess.

22 Place back the rack cover, transfer to 4˚C refrigerator, and incubate overnight

Stain 2 (1h)

23 Prepare 1 hr antibody master mix as described above in steps 15 to 18

Optional: include use anti-biotin or other anti-hapten antibodies when apply

24 Following overnight incubation, wash with 1 mL of wash buffer

25 Repeat wash by 1 mL of wash buffer

26 Add 300 µL antibody buffer (NHS 3%)

27 Make sure that the antibody buffer has passed thru to prevent dilution of the antibody mix

Add Stain 2 antibody mix and incubate for 1 hour at 4˚C

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Wash and Post-fixation

29 Add 1 mL of wash buffer

30 Repeat step 29

31 Remove the slide for the assembly

32 Immerse the slide in post-fixation buffer (glutaraldehyde 4% in PBS-low Barium) for 5 min

33 Transfer in PBS-low Barium for rinse, <2 min

Dehydration

34 Rearrange and fill additional reagent containers:

Tris buffer pH 8.5 x 3, ddH2O x 2, 70% EtOH, 80% EtOH, 95% EtOH x 2, 100% EtOH x 2, exit tank = empty and dry

35 Insert slides into slide carriers. Place slide carriers into placeholder containers into the first Tris buffer container

36 Menu --> Processing time = 30 sec, Lift bar = 976, Number of dips = 3, Start position = position of the first slide carrier

37 Press Run

38 Dry in vacuum desiccator with the slide carrier lid open for at least 1 hour prior to MIBI analysis

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