abcam

SWITCH protocol

Developed by the Chung lab

http://www.chunglab.org/

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SWITCH (system-wide control of interaction time and kinetics of chemicals) is a simple, scalable, and generalizable tissue-processing method for proteomic imaging of intact biological system. The SWITCH tissue-processing and clearing method provides access to high-dimensional multi-scale information that may help to understand health and disease from molecules to cells to entire systems.

Contents

Reagents

Reagent	Vendor
32% Paraformaldehyde (PFA)	Electron Microscopy Sciences, 15714-S
50% Glutaraldehyde (GA)	Electron Microscopy Sciences, 16310
Potassium hydrogen phthalate	Sigma-Aldrich, P1088
Acetamide	Sigma-Aldrich, A0500
Glycine	Sigma-Aldrich, G7126
Sodium azide	Sigma-Aldrich, S2002
Triton-X (TX) 100	Amresco, 0694
Sodium dodecyl sulfate (SDS)	Sigma-Aldrich, L3771
Sodium sulfite	Sigma-Aldrich, S0505
DiD	ThermoFisher, D7757
N- methyl-D-glucamine	Sigma-Aldrich, M2004
Diatrizoic acid	Sigma-Aldrich, D9268
60% iodixanol	Sigma-Aldrich, D1556

Solution preparation

Perfusion solution

This solution should be made fresh immediately prior to performing perfusion and kept on ice at all times. It is recommended to chill all of the separate ingredients before mixing the components.

Reagent	Volume
10X PBS	4 mL
32% PFA	5 mL
50% GA	0.8 mL
Water	30.2 mL

Fixation-OFF solution

Titrate a bottle of PBS to pH 3 using HCl. Create solutions of 0.1 M HCl in water and 0.1 M potassium hydrogen phthalate (KHP) in water. Finally, mix these solutions in a ratio of 2:1:1 (pH 3 PBS):(0.1 M HCl):(0.1 M KHP).

To this solution, add a stock solution of GA to make a final concentration of 4% GA. Ensure that this solution stays cold at all times. It is recommended to chill the solution before adding GA.

Fixation-ON solution

Add a stock solution of GA to PBS (pH 7.4) to make a final concentration of 1% GA. Ensure that this solution stays cold at all times. It is recommended to chill the PBS before adding GA.

PBST

To PBS, add TX-100 to a final concentration of 0.1% (v/v). Also, add sodium azide to a final concentration of 0.02% (w/v).

Reagent	Amount
1X PBS	1L
TX-100	1 mL
Sodium azide	0.2g

Inactivation solution

To PBS, add acetamide to a final concentration of 4% (w/v) and glycine to a final concentration of 4% (w/v).

Thermal clearing solution

To water, add SDS to a final concentration of 200 mM and sodium sulfite to a final concentration of 20 mM. This solution should be made fresh frequently, as the sulfites tend to degrade over time in solution.

DiD-OFF solution

To PBS, add SDS to a final concentration of 10 mM. Dissolve 1mg of DiD powder per 200 µL. This solution should be kept protected from light. Note: molecules similar to DiD can be used if other excitation/emission wavelengths are desired, so long as the molecule is sufficiently lipophilic.

Antibody-OFF solution

To PBS, add SDS to a final concentration of 0.5 mM. This is most easily accomplished by diluting a stock solution of SDS. When adding large proportions of antibody to this solution (say, >1:10), care should be taken to account for the resulting change in SDS concentration.

Optical clearing solution

This solution consists of 23.5% (w/v) N-methyl-D-glucamine, 29.4% (w/v) diatrizoic acid, and 32.4% (w/v) iodixanol in water. Use a stir bar (or shake if necessary) to fully dissolve the powders at each step. Do not use heat when mixing the solution, as this will cause a color change.

This solution should be stored carefully to ensure that no water is lost, as just a small amount of evaporation will result in precipitation. Teflon tape can be used to increase the security of the bottle's seal, and parafilm can be used around the cap.

It may be necessary to use a 60% iodixanol solution (see reagents list) rather than iodixanol powder, as it is not cheaply available. The optical clearing solution in the case would look as follows:

Reagent	Volume
47% Iodixanol solution in water*	10 mL
N-methyl-D-glucamine	3.39 g
Diatrizoic acid	4.24 g

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Method

1. All samples must be preserved by either procedures 1a or 1b, and then inactivated by procedure 2 and cleared through procedure 3, in order.

2. Procedures 4a and 4b are optional, but it is not recommended to perform both in the same round of staining.

3. Samples thicker than 50–100 µm must undergo procedure 5 in order to be imaged fully, but it is optional for very thin samples.

4. After procedure 6, you may go back to procedure 4a or 4b to complete another round of staining.

5. Processing times at each step will vary depending upon the tissue type and size of the sample.

6. Unless otherwise noted, the parameters given below were optimized for adult mouse brain samples.

1. Tissue preservation

1a. Perfusion

Perfusion is the preferred method of tissue preservation. Using the perfusion technique of your choice

- Perfuse 20 mL of ice-cold PBS through the beating heart of an anesthetized mouse, followed by 20 mL of the ice-cold **perfusion solution**.

  Take care not to introduce any bubbles during the procedure, and use a flow rate slow enough to avoid damage to the vasculature or brain sample (<5 mL/min).

- After both solutions have been perfused, carefully remove the brain from the skull using any technique you are comfortable with.

  The dura membrane should also be removed during the process.

- Place the sample into 20 mL of **perfusion solution** and incubate at 4˚C with gentle shaking for 3 days.

1b. SWITCH-mediated tissue preservation

If perfusion is not possible, the sample must be preserved using SWITCH.

- Fix the sample with PFA for several days before proceeding.
- Incubate the sample in 40 mL **fixation-OFF solution** at 4˚C with gentle shaking for 2 days.
- Transfer the sample to **fixation-ON** solution at 4˚C with gentle shaking for an additional 2 days.

Note: the timing for the **fixation-OFF** and **-ON** steps is dependent on the sample size and may need to be optimized from these starting values on a case-by-case basis. We found that these parameters worked well for banked human samples of roughly 0.5–1.0 cm thickness.

2. Fixative inactivation

After fixation via either perfusion or SWITCH, the sample must be washed in PBST to remove unbound fixative molecules.

- For mouse brains, 2 washes in **PBST** of 6 hours each at room temperature (RT) with gentle shaking is generally sufficient.
- Wash samples in **inactivation solution** at 37 ˚C overnight to inactivate remaining fixative molecules.
- If the solution turns yellow, the **inactivation solution** should be replaced with fresh solution and the sample incubated for several more hours.

Note: if the sample needs to be cut, this should take place now before the sample is cleared.

3. Thermally-assisted lipid clearing

Inactivated samples must next be incubated in **thermal clearing solution** to wash away remaining **inactivation solution** and to distribute sodium sulfite through the sample.

- Perform 2 washes in **thermal clearing solution** of 6 hours each at room temperature (RT)
- Place the samples in a tube of fresh **thermal clearing solution**

– Incubate in a water bath heated to 70 ˚C

The length of time for incubation will depend on the size of the tissue. For mouse brain sections this is approximately 2 hours for a 100 µm section, up to several days for a whole mouse brain. It is important for that the user regularly check the progress in order to gauge the correct length of time for incubation.

Other temperatures or methods of consistent heating may be used, but samples may deteriorate over time at higher temperatures.

Note: If a sample contains fluorophores that were genetically-encoded, introduced through viral injection, etc, then the sample may be cleared at 37 ˚C to preserve this fluorescence. The clearing process will take much longer at this low temperature, but temperatures higher than this will result in loss of fluorescence during clearing.

Note: Falcon tubes can become fragile over time in these conditions, so it is necessary to frequently check that the tubes have not begun to leak.

4. Labelling

4a. SWITCH-mediated myelinated fiber-labelling

After a sample has been cleared, SWITCH-mediated labelling is possible. Myelinated fibers can be readily visualized with the lipophilic DiD fluorescent molecule.

– Equilibrate samples in a solution of 10 mM SDS in PBS in order to distribute SDS molecules throughout the sample.

– Placed in a volume of **DiD-OFF solution** just large enough to cover the sample and incubate at 37˚C with gentle shaking for 12 hours to 7 days depending on the size of the sample (1 mm-thick section to whole mouse brain).

– Transfer to 40 mL of **PBST** and incubate at 37˚C for 12 hours to 2 days.

  We have also observed that tomato lectin and nuclear stains such as DAPI or Syto16 can be used with this SWITCH approach.

4b. SWITCH-mediated immunolabelling

After a sample has been cleared, SWITCH-mediated labelling is possible.

– Equilibrated the sample in **antibody-OFF** solution in order to distribute SDS molecules throughout the sample.

– Place in a fresh volume of **antibody-OFF** solution just large enough to cover the sample.

– Add antibodies in the desired quantities.

– Incubate at 37˚C with gentle shaking for 12 hours to 7 days depending on the size of the sample (1 mm-thick section to whole mouse brain).

– Transfer the sample to 40 mL of **PBST** and incubate at 37 ˚C for 12 hours to 2 days.

5. Optical clearing

After labelling, the sample must be equilibrated in a refractive index-matching solution to facilitate imaging.

- Wash the sample in **optical clearing solution** at least 3 times for 6 hours each, at 37˚C, with gentle shaking.
- After the final wash, the sample should be clear enough to easily see through by eye.
- If the solution immediately surrounding the sample seems inhomogeneous, it suggests that the sample has not yet fully equilibrated with the solution.

6. Molecular probe elution

After imaging, the **optical clearing solution** should be washed out of the sample with **thermal clearing solution**.

- After the sample has equilibrated, place the sample in a 70 ˚C water bath for 2 hours to overnight depending on the size of the sample.
- Labelling can proceed again after this step is completed.

Reference

Murray, E., Hun, J., Goodwin, D., Ku, T. & Swaney, J. Simple, scalable proteomic imaging for high-dimensional profiling of intact systems. Cell 163, 1500–1514 (2015).