LifeCanvas Technologies
1035 Cambridge St, Suite 6
Cambridge, MA 02141,USA
www.lifecanvastech.com

SHIELD Protocol with LifeCanvas Devices

ORIGINAL ARTICLE:

Young-Gyun Park, Chang Ho Sohn, Ritchie Chen, Margaret McCue, Dae Hee Yun, Gabrielle T Drummond, Taeyun Ku, Nicholas B Evans, Hayeon Caitlyn Oak, Wendy Trieu, Heejin Choi, Xin Jin, Varoth Lilascharoen, Ji Wang, Matthias C Truttmann, Helena W Qi, Hidde L Ploegh, Todd R Golub, Shih-Chi Chen, Matthew P Frosch, Heather J Kulik, Byung Kook Lim & Kwanghun Chung. Protection of tissue physicochemical properties using polyfunctional crosslinkers, ***Nature Biotechnology***, 2018 Dec 17, DOI: 10.1038/nbt.4281

REAGENTS:

SHIELD-Epoxy Solution (SH-ES) - Store at 4°C upon delivery.
SHIELD-Buffer Solution (SH-BS) - Store at RT
32% Paraformaldehyde Solution (15714-S Electron Microscopy Sciences)
SHIELD-ON Buffer(SH-ON) - Store at 4°C upon delivery.

If your experiment allows it, the best preservation technique involves perfusion of the live animal. If you cannot perfuse your animal or are working with PFA-fixed tissues, it is possible to post-fix your sample with SHIELD. If you will be perfusing, please proceed to the **PERFUSION PROTOCOL**, and if you will be post-fixing a sample that is already fixed with PFA, please proceed to **POST-FIXATION PROTOCOL**. If your sample is PFA-fixed human tissue, please proceed to **POST-FIXING PFA-FIXED HUMAN SAMPLES** and if your sample's shortest length scale is <1.5mm please proceed to **PRESERVING SMALL SAMPLES.**

PERFUSION PROTOCOL

PROCEDURE:

1. Prepare SHIELD Perfusion Solution **fresh** on ice (see **REAGENT SETUP**), and transcardially perfuse the animal with ice-cold PBS followed by ice-cold SHIELD Perfusion Solution in the following volumes and flow rates. Keep the remaining SHIELD Perfusion Solution on ice for use in Step 2.

Step 1	PBS (mL)	SHIELD Perfusion Solution (mL)	Flow Rate (mL/min)
Mouse	20	20	5
Rat	200	200	60

   - Perfuse with PBS until the fluid is running completely clear before perfusing with SHIELD Perfusion Solution.
   - Be careful not to introduce air bubbles inside tubing.
   - When the fluid comes out of the mouth or a lung swells, adjust the position of needle in the heart.

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2. Dissect out and incubate the brain in the remaining SHIELD Perfusion Solution at 4°C with shaking. Make sure the brain is immersed in the solution during shaking. Use the following volumes and incubation times:

Step 2	SHIELD Perfusion Solution Volume (mL)	Incubation Time (day)
Mouse	20	2
Rat	50	2

We recommend cutting the brain into hemispheres with a razor blade after this step. If your study requires an intact whole-brain, you do not need to cut it.

3. Prepare fresh SHIELD OFF Solution (see **REAGENT SETUP**) and incubate at 4°C with shaking. Use the following volumes and incubation times:

Step 3	SHIELD OFF Solution Volume (mL)	Incubation Time (day)
Mouse	20	1
Rat	50	3

4. Transfer the brain to SHIELD ON Buffer (RT) and incubate at 37°C with shaking:

Step 4	SHIELD ON BUFFER Volume (mL)	Incubation Time (hr)
Mouse	20	24
Rat	40	24

SHIELD preservation is now complete. The sample can be stored in 1X PBS with 0.02% sodium azide at 4°C for several months without significant loss of fluorescence signal and structural integrity.

5. You may now proceed to the **TISSUE CLEARING** section of the protocol.

POST-FIXATION PROTOCOL

Note: if you followed the **PERFUSION PROTOCOL**, this protocol is not necessary. If your sample is PFA-fixed human tissue, please proceed to **POST-FIXING PFA-FIXED HUMAN SAMPLES** and if your sample's shortest length scale is <1.5mm please proceed to **PRESERVING SMALL SAMPLES**.

PROCEDURE:

1. Prepare SHIELD-OFF Solution according to the **REAGENT SETUP** and incubate the sample in it at 4°C with shaking for 1 days, 4 days, or 6 days for thin slices, mouse brain hemisphere or rat brain hemisphere respectively.

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2. Transfer the brain to SHIELD-ON Buffer (RT). Use the same volume of solution as **Step 1**. Incubate the sample at 37°C with shaking for 24 hours.

3. SHIELD post-fixation is now complete. The sample can be stored in 1X PBS with 0.02% sodium azide at 4°C for several months without significant loss of fluorescence signal and structural integrity.

4. You may now proceed to the **TISSUE CLEARING** section of the protocol.

REAGENT SETUP

REAGENT SETUP:

SHIELD Perfusion Solution (prepare fresh before perfusion)

Mix the following **in the order shown in the table** and keep on ice. After adding each reagent, please vortex or mix well to prevent precipitate formation. This is the total volume required for the entire **PERFUSION PROTOCOL** (Steps 1 and 2).

Reagent	For 1 Mouse (40 mL total)	For 1 Rat (250mL total)
DI Water	5	31.25
SHIELD-Buffer Solution	10	62.5
32% Paraformaldehyde Solution	5	31.25
SHIELD-Epoxy Solution	20 **(add in 10 mL increments)**	125 **(add in 25 mL increments)**

SHIELD-OFF Solution (prepare fresh for Step 3 of **PERFUSION PROTOCOL** or Step 1 of **POST-FIXATION PROTOCOL**)

Mix the following **in the order shown in the table** and keep on ice. After adding each reagent, please vortex or mix well to prevent precipitate formation.

Reagent	For 1 Mouse (mL)*	For 1 Rat (mL)*
DI Water	5	12.5
SHIELD-Buffer Solution	5	12.5
SHIELD-Epoxy Solution	10	25

* If you are post-fixing a sample, this volume is for mouse brain, rat brain, or similarly sized tissues. SHIELD works with a wide variety of tissues, but the total volume of solution required is dependent on tissue size.

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POST-FIXING PFA-FIXED HUMAN SAMPLES

PROCEDURE:

1. Prepare SHIELD-OFF Solution according to the **REAGENT SETUP** and incubate the sample in it at 4°C with shaking for 2 days for 1 mm thick slices. Thicker slices may require longer incubation.

2. In a 50 mL conical tube, mix SHIELD-ON Buffer and SHIELD-Epoxy Solution in a ratio of **1:1**. Use the same volume of solution as **Step 1**. Incubate the sample at 20°C with shaking for 1 day.

3. SHIELD post-fixation is now complete. The sample can be stored in 1X PBS with 0.02% sodium azide at 4°C for several months without significant loss of fluorescence signal and structural integrity.

4. You may now proceed to the **TISSUE CLEARING** section of the protocol.

PRESERVING SMALL SAMPLES

This protocol should be used for any sample with its smallest dimension 1.5 mm or smaller. It can be used for drop-fixation of mouse spinal cords.

PROCEDURE:

1. If your sample can be perfused, proceed through **Step 2** of the **PERFUSION PROTOCOL** and then continue with this protocol. If your sample will be post-fixed, proceed through **Step 1** of the **POST-FIXATION PROTOCOL** and stop there before continuing with this protocol.

2. In a 50 mL conical tube, mix SHIELD-ON Buffer and SHIELD-Epoxy Solution in a ratio of **7:1**. The total volume needed will be about 20 mL**.** Incubate the sample at 37°C with shaking for 3-6 hours. This time is dependent on tissue size.

3. Transfer the sample to a new conical tube with the same volume of fresh SHIELD-ON Buffer (containing NO SHIELD-Epoxy Solution) and incubate at 37°C with shaking overnight.

4. SHIELD post-fixation is now complete. The sample can be stored in 1X PBS with 0.02% sodium azide at 4°C for several months without significant loss of fluorescence signal and structural integrity.

   You may now proceed to the **TISSUE CLEARING** section of the protocol.

TISSUE CLEARING

SHIELD-processed tissues can be cleared both passively with EasyClear (or temperature controlled shaking water bath) or actively with SmartClear II Pro.

ACTIVE CLEARING (SMARTCLEAR II PRO)

PROCEDURE:

Install buffers and membranes in the SmartClear II Pro system. After washing the sample in PBS, carefully place the tissue in the sample holder and put it in the SmartClear II Pro system. For optimal clearing, align the long axis of your sample vertically in the holder. Buffer A temperatures of 40-45°C can clear a mouse brain hemisphere preserved with perfusion in 3-4 days, yielding better retention of fluorescence signal than passive clearing. Post-fixed samples may take longer to clear. For FISH experiments, it is best to clear at 37°C for 7-8 days. Higher temperature will lead to significant loss of RNAs. The sample will not appear transparent, but rather translucent. Samples will become transparent after **INDEX MATCHING (EASYINDEX).** Samples that are not fully cleared will show an opaque center.

PASSIVE CLEARING

REAGENT SETUP:

SDS Clearing Solution:

Prepare the following solution in DI water. These are just suggested vendors, you can use any vendor you would like:

Reagent	Vendor	Product Number	Final Concentration (mM)
Sodium dodecyl sulfate	Sigma-Aldrich	75746	300
Boric acid	Alfa Aesar	12680	10
Sodium sulfite	Sigma-Aldrich	S0505	100
Sodium hydroxide	Sigma-Aldrich	S5881	Titrate to pH 9

PROCEDURE:

Incubate the sample at 37-60°C with shaking. Higher temperatures will clear tissue faster but result in smaller retention of fluorescence signal. It will take 8-14 days to clear a mouse brain hemisphere at 45°C. For FISH experiments, it is best to clear at 37°C. The sample will not appear transparent, but rather translucent. Samples will become transparent after **INDEX MATCHING (EASYINDEX).** Samples that are not fully cleared will show an opaque center.

INDEX MATCHING (EASYINDEX)

PROCEDURE:

Place the tissue in 20-40 mL of EasyIndex and incubate in a shaker at RT until the sample is evenly translucent or haze is not visible in the solution any more. Incubation time is proportional to the size of the tissue, usually 24 hours for a mouse brain hemisphere in 40 mL of the solution. The volume of the solution can be adjusted by the size of the tissue.