

X-Clarity PRESTO Protocol

- 1- Perfusion with 20-50mL of PBS, then 20-50mL 4% PFA
- 2- 24h at 4°C in 4% PFA (1 volume of organ to 10 volume of fixative).
- 3- Wash in PBS at RT
- 4- 24h at 4°C in bis-free hydrogel (1 volume of organ to 10 volume of hydrogel) with agitation.
It would be better to use a flat bottom container.

HYDROGEL SOLUTION

Product used	Product description	Supplier & cat. n°	Final conc.	Quantity for 200ml final volume	Purpose
PBS 10x	-	SV solution pH6.8	1x	20ml	Salt buffer
40% Acrylamide bis-free	CAS: 79-06-1 MW= 71.08g/mol	Sigma A4058-100ML	4%	20ml	Hydrogel network monomer
VA-044 initiator	CAS: 27776-21-2 MW= 323.33g/mol	IGZ 017-19362	0.25%	0.5g	Polymerisation thermal initiator
MilliQ H ₂ O	CAS: 7732-18-5 MW= 18.02g/mol	-	-	160ml	Aqueous solvent

Solution can be stored at -20°C in aliquots but not at 4°C for more than 48 hours.

The clearing of an entire brain takes only 6-8 hours (check sample every 2 hours) compared to 24-48 hours with PFA in the hydrogel. It can be as well useful for sensitive GFP signal.

- 5- Polymerization: 3h, 37°C, O₂-free

Just take the tube and put it at 37°C (water bath is better than oven). O₂-free is important but it is not a big deal: just fill up the whole tube with hydrogel and close it, so that oxygen can't be present. The acrylamide around does not form a compact gel but becomes viscous.

- 6- Wash the sample 1-2h in PBS
- 7- The sample can be stored in PBS at 4°C waiting for the clarification step (make slices at the step if wanted).
- 8- ETC in X-CLARITY using SDS buffer

CLEARING SOLUTION

Product used	Product description	Supplier & cat. n°	Final conc.	Quantity for 2.5L final volume	Purpose
Boric acid	CAS: 10043-35-3 MW= 61.83g/mol	Sigma B7901-1Kg	0.2M	30.9g	pH buffer
NaOH	CAS: 1310-73-2 MW= 40g/mol	Sigma 71960	-	To pH 8.5	pH adjustment ions
SDS dust free pellets	CAS: 151-21-3 MW= 288.38g/mol	Sigma 75746-1KG	4%	100g	Lipid clearing surfactant
MilliQ H ₂ O	CAS: 7732-18-5 MW= 18.02g/mol	-	-	Up to 2.5L	Aqueous solvent

Dissolve the boric acid (g) in 1 Liter of Water and adjust the pH to 8.5 with NaOH pellets.

Add the SDS pellets progressively and complete with water to 2.5 Liters. It is possible to buy 20% liquid SDS as well.

SETUP

Fill the bottle with 1.2L of SDS buffer and do not forget to change the solution after 12 hours.

Fill the chamber until the metal bar (CW rotation of the pump).

Place the sample in the chamber (note the orientation of the chamber).

Close the cover tightly to avoid leakiness.

Fill the chamber completely.

Start the current.

Current	1.5 A
Temperature	37°C
Switching time	0 min
Pump speed	30 rpm

To control the clearing, stop the current.

Empty the chamber until the metal bar (CCW rotation, do not use full speed).

Take out the sample to control clearing and place it back or continue to step 9.

9- 24h in PBST (0.1% Triton X-100 in 1x PBS) to completely remove the SDS, at 37°C or RT with shaking (never at 4°C!).

10- The sample can now be stored in PBS at 4°C, waiting for immunostaining or imaging of endogenous fluorescence reporter

11- For acquiring images in the presence of Histodenz medium, follow this protocol:

- The sample is immersed in 30 ml of Histodenz medium in a 50ml Falcon tube, with a slow shaking in the dark for at least 3 hours at RT, and if the sample is more than 0.5 cm³ then exchange the medium with 30 ml of fresh Histodenz medium and incubate for 2 hours more. The sample can be kept in Histodenz medium for 24 hours. After these two hours additional incubation in Histodenz medium, check if the refraction index of the Histodenz medium is at RI 1.45, if not change the environment with 30 ml of fresh Histodenz medium.
- 30ml Histodenz medium are needed to fill a clearing chamber of sheet Light Microscope Z.1 (Zeiss)

Phosphate Buffer (PB) SOLUTION

Product used	Product description	Supplier & cat. n°	Final conc.	Quantity for 1000 ml final volume
Sodium dihydrogen phosphate	NaH ₂ PO ₄ · H ₂ O CAS : 10049-21-5 MW=137.99g/mol	Sigma 71507	0.24M	6.4 g
di-sodium hydrogen phosphate anhydrous	Na ₂ HPO ₄ CAS : 7558-79-4 MW=141.96g/mol	Sigma 71642	0.24M	27.0 g

IMAGING SOLUTION

Product used	Product description	Supplier & cat. n°	Final conc.	Quantity
0.24M PB	-	Labmade (see above)	0.02M	6ml
MilliQ H ₂ O	CAS: 7732-18-5 MW= 18.02g/mol	-	-	54ml
Tween 20	CAS: 9005-64-5	Sigma 93773	0.1%	60ul
6% NaN ₃	CAS: 26628-22-8 MW= 65.01g/mol	Stock solution in water Labmade	0.01%	100ul
NaOH	CAS: 1310-73-2 MW= 40g/mol	Sigma 71960	To pH 7.5	-

Prepare the 60ml solution, add 73g of Histodenz (Sigma D2158) and dissolve under agitation. The final volume is around 90ml and RI 1.45.