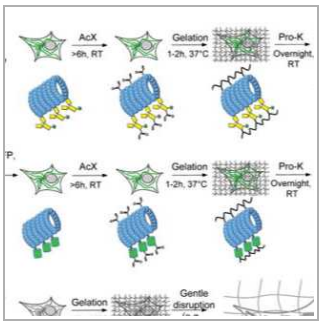

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WORKS FOR ME 1

ProExM

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monica.fernandez-monreal[1]

[1]Bordeaux Imaging Center

monica.fernandez-monreal: Modified from Ed Boyden and Paul Tillberg

monica.fernandez-monreal

COMMENTS 0

ABSTRACT

Classical protocol of protein pre-labeling Expansion- ProExM
(c) Ed Boyden

PROTOCOL CITATION

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Solutions

1 Gel and disgestion

Step 1 includes a Step case.

Monomer solution

Digestion solution

Gelling

step case

Monomer solution

A	B	C	D
Component	**Stock concer**	**Final concen**	**Amount**
Sodium acrylate	38 %	8.6 %	2.25
Acrylamide	40 %	2.5 %	0.625
N,N'-Methylenebisacrylamide	2 %	0.15 %	0.75
Sodium chloride	29.2 %	11.7 %	4

A	B	C	D
PBS	10x	1x	1
Water			0.775
Total			**9.4**

Can be aliquoted and kept at -20ºC up to 2-3 weeks.

2 Fixation
Step 2 includes a Step case.
Fixative

16h

Fluorescence Labeling

1h

step case

Fixative

Rat Hippocampal Neurons
4% Paraformaldehyde
4% Sucrose
In PBS

3 **Fixation:** Use fresh 4% PFA solution in PBS (w/ or wo/ 4% sucrose) for 15-20 min. Wash 3 times in PBS and quench aldehydes with glycine or sodium borate.

1h

4 Permeabilize in blocking buffer (0.2% fish gelatin, 0.1% or 0.3% triton in PBS), 1 hr at RT.

12h

5 Incubate with primary antibodies in blocking buffer 1h at RT, or overnight at 4ºC.

30m

6 Wash slices with blocking buffer, 4 times, ~10 min each.

1h

7 Incubate slice with secondary antibodies in blocking buffer 1 h at RT on a shaker.

8 Wash with PBS, 3 times, ~10 min each. 30m

Anchoring 6h 30m

9 Dilute AcX 1:100 (0.1 mg/mL) in 1x PBS (prepare 500 uL/well for 12-well plates).

10 Wash 2x 15 minutes with PBS before proceeding to gelation. Samples can be stored at 4º C. 30m

11 Treat stained expressing genetically encoded FPs slices or coverslips for > 6 hours at RT (this reaction can be left overnight). 6h

Gelling 2h 40m

12 Make sure to remove excess PBS from brain slices before incubation with gelling solution. Incubate slices in gelling solution in a 24-well plate for 25 min at 4C. Coverslips with cells can be mounted directly. 25m

13 Create a humid gel chamber. For 50 um slices, 2 coverglass were used to create a 170 um-depth sandwich chamber. Cell cultures were mounted cells side-down over a drop of gel (35 um for a coverslip of 12 mm). Make sure the slices are flat, and avoid air bubbles trapped inside the chamber. 15m

14 Incubate 1h or 2h at 37ºC. 2h

Digestion and expansion 13h

15 Cover the gel in digesting buffer for 15 min and add proteinase K for and overnight incubation @ room temperature (make sure at least 10-fold excess volume of digestion buffer is used, and make sure make it does not dry out). 12h

16 Wash slices with excess volume of ddH2O (we usually use at least 10x the final gel volume), 3-5 times, for 15mins each time. Slice expansion reaches plateau after about the 3rd or 4th wash. The expansion chamber needs to be of adequate size for the sample: hemislices of mouse brains fit nicely in a 6-well plate when the excess gel around the brain is trimmed away (a razor blade works well for this). 18 mm coverslips can be punched in 4 mm medals before expansion to get gels of around 16 mm. 1h

Mounting the gel 35m

17 Use a cleaned coverslip and add poly-L-lysine 0.1% solution to the top surface for 20-30 min. Rinse with ddH2O and air dry. 30m

18 Remove excess of liquid around the gel and place the expanded sample on the coverslip. After 20-30 sec, add ddH2O to keep the sample hydrated. 5m