

Fixation procedure for mouse embryos for immunofluorescence labeling

Mouse embryos are dissected in cold PBS and then fixed in 4% PFA (in PBS) at +4°C, or on ice according to the following procedure:

Developmental stages	Fixation Times	PBS washes (at 4°C)	Cryoprotection Times	Freezing Temperature
e9.5	10'	3x15'	1h30'	-55°C
e10.5	15'	3x15'	2h~3h	-55°C
e11.5	15'	3x15' + O/N	4h	-55°C
e12.5	25'	3x30' + O/N	ON	-55°C
e13.5	30'	3x30' + O/N	ON	-55°C
e14.5*	35'	3x30' + O/N	ON	-55°C
e15.5	ND	3x30' + O/N	ND	ND
e16.5*	45'	3x1h + O/N	ON	-55°C
e17.5	ND	3x2h + O/N	ND	ND
e18.5*	45'	3x2h + O/N	ON	-55°C

*Fixation in 4% PFA + 0.02% NP40 at +4°C or on ice.

For P0 to adult stages, mice are perfused intracardiacally with NaCl 9‰ or PBS and then perfused with cold 4% PFA (in PBS). The spinal cords are dissected and washed thrice in cold PBS (3X15mins), before an overnight final wash in cold PBS.

Embryos later than e11.5 are decapitated in cold PBS before fixation. After fixation, embryos are washed thrice in cold PBS (at +4°C) then a final wash overnight in cold PBS (only for stages later than e10.5).

Fixed and washed spinal cords or whole embryos are infiltrated overnight with 30% Sucrose in cold PBS. Spinal cords or embryos can be embedded in 7.5% Gelatin 15% Sucrose in PBS or in OCT and frozen at -55°C in isopentane bath before storage at -80°C.

Laboratory of Neural Differentiation - NEDI

Institute of Neuroscience - Université catholique de Louvain

avenue Hippocrate, 55 (box B1.55.11) / Harvey 55 - 3d floor

B-1200 Brussels, Belgium

Website : <http://sites.uclouvain.be/nedi>

Tel : +32.2.764.55.71 or 55.72 or 55.73; Fax : +32.2.764.55.72

Email : Frederic.Clotman@uclouvain.be