

Fixation procedure of chicken embryos for immunofluorescence labeling

Chicken 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	Cryoprotection Times	Freezing Temperature
e2.5	15'	1h30'	-55°C
e3.5	20'	2h~3h	-55°C
e4.5	30'	4h	-55°C
e5.5	45'	ON	-55°C
e6.5	1h	ON	-55°C

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 e3.5.

Fixed and washed embryos are cryoprotected in 30% Sucrose in PBS (according to the table above) before embedding in 7.5% Gelatin 15% Sucrose in PBS. Finally, blocks of embryos can be frozen at -55°C in isopentane bath and stored at -80°C.

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