

## **Fixation procedure for chick embryos for immunofluorescence labeling**

Chick 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:

<b>Developmental stages</b>	<b>Fixation Times</b>	<b>Cryoprotection Times</b>	<b>Freezing Temperature</b>
<b>e2.5</b>	15'	1h30'	-55°C
<b>e3.5</b>	20'	2h~3h	-55°C
<b>e4.5</b>	30'	4h	-55°C
<b>e5.5</b>	45'	ON	-55°C
<b>e6.5</b>	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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