Mouse Tissue Fixation with Paraformaldehyde for Fluorescent Reporter Mice

Version 2

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ABSTRACT

Fixation of mouse tissues for either histology or fluorescence microscopy is an essential first step in tissue preparation. In this protocol, we outline a reliable method for fixation and perfusion of mice for tissue histology or microscopy. Tissues prepared by this method can be used downstream as FFPE sections, cryosections for fluorescence microscopy, or tissue clearing for whole tissue imaging. For mice with transgenic fluorescent reporters, it is especially important that the tissues are preserved properly to ensure that the signal of the fluorescent reporter can be imaged.

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

MATERIALS

NAME	CATALOG #	VENDOR
50 ml conical tubes		
PBS 1X		
EDTA	AM9261	Invitrogen - Thermo Fisher
10mL syringe	75846-756	VWR international Ltd
21G needle	BD-305165	VWR international Ltd
Forceps (Dumont)	FC-5043	Braintree Scientific
Forcep (Adson)	FC02 8	Braintree Scientific
Surgical scissors (Iris - straight)	SCT-I 528	Braintree Scientific
Paraformaldehyde 32% (methanol free)	15714	Electron Microscopy Sciences

1 Prepare 1.6% PFA in PBS (at least 20 ml per mouse, half to be used during perfusion, half to be used as storage buffer overnight) MAKE THIS FRESH, DO NOT USE ANYTHING OLDER THAN 24 HOURS. Also, make sure PFA has been stored away from light, this will degrade it.

2 Prepare PBS with 2mM EDTA (at least 10 ml per mouse).

3 Sacrifice the mouse with CO_2. As soon as the mouse has stopped breathing, begin the procedure.

4 Dissect open the thoracic cavity, taking care not to puncture the heart with the scissors or forceps.

5 Fill a 10mL syringe with **10 ml PBS with 2mM EDTA**, attach needle to syringe.

6 Insert needle into mouse's right ventricle (left side of heart when looking at the dissected mouse from your perspective). Slowly perfuse mouse until lungs turn white and liver has turned brown (instead of red).

7 Fill the syringe with **10 ml 1.6% PFA in PBS**. Repeat perfusion. Mouse should appear stiff at the end of this perfusion.

8 Remove tissue of interest carefully so that marks are not made in the tissue.

9 Place tissue in **10 ml remaining 1.6% PFA in PBS** in either 15 or 50mL conical (depending on how large the tissue is).

10 For larger, thicker tissues, leave at room temperature overnight (**16:00:00** - **20:00:00**) in a dark place or covered well in foil. For smaller tissues (i.e. lymph nodes) or for tissues that require antibody staining, fix at room temperature for a few hours (2-8, depending on downstream application).

11 Wash with PBS 1x the next day, 3 times. Use at least **10 ml** (or more) to wash the tube with the fixed tissue in it. Let tissue sit in fresh PBS wash about **00:20:00** each time in Place in **4 °C** until ready to use.