Acrolein + PFA perfusion for immunocytochemistry

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Abstract

Appropriate tissue fixation is essential for a good quality immunocytochemistry (ICC).

There are several fixation methods, but "whole body" or "target" perfusion of the animal is one of the most efficient methods.

Most perfusing protocols include only paraformaldehyde, but acrolein penetrates tissue more rapidly, improving final fixation of the tissue.

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Protocol

Step 1.
Prepare animal as for Paraformaldehyde fixation; e.g. animal anesthetized with Nembutal, open chest cavity; inject 0.1 cc heparin into the heart apex before first rinse. We clamp the descending aorta to eliminate whole body perfusion. Cut atrium.

Step 2.
Saline nitrite rinse until clear effluent; about 150-200 ml/225-250 g rat.

AMOUNT
1000 ml : 0.9% saline
AMOUNT
20 g : Sodium Nitrite

Step 3.
Switch to Acrolein/Paraformaldehyde; continue until animal is hard, as judged by the front feet. Requires approximately 150-200 ml/225-250 g rat.

AMOUNT
500 ml : 8% PFA
AMOUNT
100 ml : 0.5 M Monobasic Potassium Phosphate
AMOUNT
100 ml : 0.5M Dibasic Potassium Phosphate
AMOUNT
275 ml : destilled water
AMOUNT

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25 ml : acrolein

SAFETY INFORMATION

add acrolein just before ready to perfuse; if using a sealed bottle or flask, slowly release pressure after doing so and before perfusing

Step 4.

Second saline nitrite rinse; ≈150 ml. No post-fixation necessary.

Warnings

Acrolein is very toxic, similar to tear gas, so wear mask and don't get too close!

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