

PFA Fixation for Immunofluorescence

** This fixation protocol works well for most antibodies and fusion proteins. We use it at 37°C to preserve microtubules in mitotic cells, but it can also be used at room temperature. The time of fixation will vary according to your particular antibody/protein, but 10 minutes is a good starting point.*

2X PHEM Buffer (500 mls)

18.14 g PIPES
6.5 g HEPES
3.8 g EGTA
0.99 g MgSO₄
pH to 7.0 with 10M KOH

Note: will not go into solution until pH approaches 7!!!
Filter and store in frozen aliquots or at 4°C.

37% PFA (5 mls) ** make fresh every time!

1.85 g PFA
3.5 ml dH₂O
10 ul 10M KOH

Mix together in a 50 ml Falcon tube. Boil water in a glass beaker in the microwave and put tube in it with cap loose, swirling frequently to mix, for no longer than 5 minutes, until the PFA goes into solution.

Fixation Buffer (50 mls)

25 ml 2X PHEM buffer
5 ml 37% PFA
20 ml dH₂O

Warm to 37°C and add directly to cells after pouring media out of the dish. Fix for 5-20 minutes (10 minutes is a good average).

Following this fixation cells will still need to be permeabilized prior to antibody staining. We use 1% Triton X-100 in PBS, and NP-40 works as well. This is usually done for 10 minutes, with further PBS washes afterwards.