

<http://www.abrn.net/pdf/Formalin%20fixation%20protocol%20August04.pdf>

*1 mm penetration of formalin per hour for first hour, then 1mm penetration per 3 hours for subsequent thickness at room temperature.*

<http://www.histosearch.com/histonet/May04/Re.HistonetCD10CD103FDAfa.html>

The penetration rate of formaldehyde is largely irrelevant to tissue no more than 1.5 cm thick.

However, it's NOT 2mm per hour and fixation takes much longer than this!

It's variable.  $d = K \times \text{the square root of time.}$

K = the coefficient of diffusibility.

Baker determined  $K=3.6$ , probably correct and a figure most people accept.

Helander's 1994 data suggests it is at least 2.0 and most likely close to 3.6.

Using  $K=3.6$  in the above formula, the first layer of cells is penetrated at a rate of over 90 mm per hour.

This slows to 3.6 mm at 1 hour.

It then takes 4 hours to double the depth penetrated to 7.2 mm, a rate of 1.8mm per hour.

At 16 hours, the depth is again doubled to 14.4 mm, a rate of 0.9 mm/hr.

i.e. to double the depth of penetration you must allow 4X the time and so doing halves the rate!

Your 4mm tissue block would be fully penetrated in less than 1 hour (3.6 mm from each surface)

What the issue really is, relates to the binding time necessary for formaldehyde.

A 90% threshold binding, i.e. MINIMAL binding time is 24 hours at 22C and 18 hours at 37C.

It doesn't matter if your tissue is only a few cells thick or 4mm thick!

16 hours is not long fixation, 3 weeks may be, and is certainly NOT overkill.

The weak initial binding of formaldehyde is freely reversed, 50% in about 12 hours, by the lower alcohols on the processor and the tissue becomes fixed by alcohol.

Any thing less than 8 hours formaldehyde fixation reduces the IHC for ER demonstrably and HER2 markedly.

<http://publish.uwo.ca/~jkiernan/FixAnti1.pdf>

References from this publication are listed –

## **References**

### **Preservation and retrieval of antigens for immunohistochemistry – methods and mechanisms. 1. Effects of formaldehyde fixation.**

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