Elimination of mercury salts from tissue sections

WOLF D. KUHLMANN

Division of Radiooncology, Deutsches Krebsforschungszentrum, D-69120 Heidelberg
Laboratory Diagnostics & Cell Science, 56112 Lahnstein

The use of sublimate in tissue fixation (e.g. Zenker solution) will lead to irritating salt precipitations in histological preparations which must be eliminated prior to microscopy. The elimination procedure is called "De-Zenkerization" and can be performed with tissue blocks just after fixation and prior to paraffin embedding or with deparaffinated tissue sections (e.g. prior or after immunostaining). *

De-Zenkerization of tissue sections (iodine/iodide in ethanol)

Chemicals	**Chemical solution**
Iodine (I_2 crystals, iodine sublimed) Potassium iodide (KI) Sodium thiosulfate Ethanol Distilled water	• Iodine/iodide stock solution: 2.0 g iodine crystals *plus* 3.0 g potassium iodide dissolved in 100.0 mL 90% ethanol (Lugol's Iodine) • Iodine/iodide working solution by method A: 1.0 mL iodine/iodide stock *plus* 99.0 mL 70% ethanol or prepare Iodine/iodide working solution by method B: add enough drops of iodine/iodide stock to 70% ethanol until the solution becomes ruby-brown colored (like cognac) • Sodium thiosulfate solution: 0.5 g sodium thiosulfate dissolved in 200 mL distilled water

* The used chemicals can be toxic. They must be handled with care

De-Zenkerization prior to immunohistology (alternatively after immunostaining)

– deparaffinize sections (xylene or xylene substitute)	2 x 5 min
– absolute ethanol	2 x 5 min
– 95% ethanol	2 x 1 min
– 70% ethanol	2 x 1 min
– Iodine/iodide working solution (method A or method B)	10-15 min
– 70% ethanol	2 x 2 min
– distilled water	2 x 5 min
– sodium thiosulfate solution	2 x 1 min
– distilled water	3 x 5 min

– perform immunohistological incubations and counterstain if desired

After immunostaining, slides are dehydrated in ascending series of ethanol, cleared in xylene or xylene substitute and mounted in resinous medium under coverglass

De-Zenkerization of tissue sections (iodine/iodide in water)

Chemicals	**Chemical solution**
Iodine (I_2 crystals, iodine sublimed) Potassium iodide (KI) Sodium thiosulfate Ethanol Distilled water	• Iodine/iodide aqueous solution: 1.0 g iodine crystals *plus* 2.0 g potassium iodide *mix and add* 5.0 mL distilled water swirl until dissolved and add 295.0 mL distilled water (Gram's Iodine) • Sodium thiosulfate solution: 3.0 g sodium thiosulfate dissolved in 100 mL distilled water

De-Zenkerization prior to immunohistology (alternatively after immunostaining)

– deparaffinize sections (xylene or xylene substitute)	2 x 5 min
– absolute ethanol	2 x 5 min
– 95% ethanol	2 x 1 min
– 70% ethanol	2 x 1 min
– distilled water	2 x 2 min
– Iodine/iodide aqueous solution	5 min
– Distilled water	several rinses
– sodium thiosulfate solution	until section is bleached
– distilled water	3 x 5 min
– perform immunohistological incubations and counterstain if desired	

After immunostaining, slides are dehydrated in ascending series of ethanol, cleared in xylene or xylene substitute and mounted in resinous medium under coverglass

In order to avoid possible deleterious effects of iodine on the detection of antigens, De-Zenkerization can also be done when all immunostainings have been performed.

References for further readings

Mayer P (1918)
Romeis B (1968)
Bancroft J, Cook HC, Turner DR, eds. (1994)

Full citation of publications is given in chapter *References*
link: https://www.kuhlmann-biomed.de/wp-content/uploads/2020/12/References.pdf

 10.09.2006