



Immunohistochemistry on FFPE tissue

Health and Safety hazards and control measures

- Hazardous chemical - Xylene



Xylene is a Flammable. Irritating to the skin, eyes, mucous membranes, and respiratory tract. A carcinogen that may damage the liver and kidneys through prolonged/repeated exposure. Acute toxicity. Wear either nitrile gloves or xylene resistant gloves. Always use in a chemical fumehood. Do not wash into drains, dispose via chemical waste route. Store in fume cabinet separate to flammable solvents. Note: if nitrile gloves are used, use forceps to minimise exposure to xylene.

- Hazardous chemical - Ethanol



Ethanol is a highly flammable liquid and vapour. It causes serious eye irritation. Wear nitrile gloves. Low risk of eyesplash as ethanol steps are performed in a fumehood. Avoid use near flames, heat or sparks.

- Hazardous chemical – H₂O₂ (30%)



Hydrogen Peroxide is a corrosive liquid that causes severe skin irritation and possible burns. It is harmful if swallowed, causes serious eye damage and is harmful to aquatic life. It is a strong oxidiser which must be stored away from combustible materials. Wear nitrile gloves and use in a chemical fumehood when making up working solution for this protocol.

- Hazardous chemical – 3,3'-Diaminobenzidine (DAB)



DAB may cause cancer and is suspected of causing genetic defects. It's a flammable liquid and causes eye irritation. Wear nitrile gloves. Do not use if pregnant. This protocol uses DAB in a commercial kit, the DAB is used in small amounts and diluted, therefore it may be used on the bench in a well-ventilated area. Dispose of surplus chemical via college chemical waste route.

- Hazardous chemical – DPX mountant



DPX is a flammable liquid that is harmful by inhalation and in contact with skin. It is irritating to the skin. It may cause harm to the unborn child and is suspected of damaging fertility. It is very toxic to aquatic organisms. Do not wash into drains, dispose via chemical waste route. Wear nitrile gloves and use only in a chemical fume hood. Do not use if pregnant. (reproductive toxicity hazard)



- Hazardous chemical - Mayer's haematoxylin solution



Mayer's haematoxylin solution is harmful if swallowed or inhaled. Use in a chemical fumehood. Avoid generation of vapours or aerosols. Wear nitrile gloves. Do not let product enter drains. Dispose of surplus chemical via college chemical waste route.

- Hazardous chemical - PAP pen



PAP pen contains liquid and vapour that is highly flammable. It may be fatal if swallowed and enters airways. It causes skin irritation. It may cause drowsiness or dizziness, genetic defects and cancer. It may cause damage to organs through prolonged or repeated exposure. It is suspected of damaging the unborn child. Do not use if pregnant. Wear nitrile gloves. Dispose empty containers containing residue chemical waste route. (*reproductive toxicity hazard*)

- **Steamer** – Hot steam may cause burns. Heat may cause glass containers to break. Staff/students are trained in the safe use of the steamer. Only use plastic containers in the steamer.
- **Covid-19** – Risk of contracting Covid-19 in the workplace. Clean touch points of equipment before and after use. Social distancing rules should be followed.

IHC on FFPE tissue protocol:

- **Incubate slide @ 60°C O/N prior to beginning protocol.**
 - **If doing IHC to optimise antibodies intended for IMC, use EDTA pH.9 instead of citrate buffer (CB)**
 - **+ve control for rabbit antibodies: GFAP**
 - **+ve control for mouse antibodies: HLA-DR**
 - **+ve control for goat antibodies: Goat anti-IBA1**
1. **In fumehood** - Dewax and dehydrate slides: x 2 in xylene, x2 100% ethanol, 90%eth, 70%eth, dH₂O, 5 mins each.
 2. Peroxidase quenching in 1:30 H₂O₂ to 1XPBS, 30 mins (10ml 30% H₂O₂:290ml PBS).
 3. During quench step, place CB in steamer with a foil lid to warm (30ml 10X stock citrate buffer: 270ml H₂O).
 4. After quench, wash slides in dH₂O wash, 5 mins.
 5. Place slides in warm 1X CB, 20 mins, then cool on ice (15 mins -~ until slides @ RT).
 6. Wash slides in x1 dH₂O, and x1 1xPBS, 5 mins each.
 7. Draw around tissue with PAP pen (*reproductive toxicity hazard*) (ab2601).
 8. Block in blocking serum if necessary.
 9. Add ~150µl of the primary antibody (1° AB in PRIMARY DILUENT (PBS-Tx 0.3%)).
 10. Incubate at RT for 1HR or 4°C overnight.
 11. 1xPBS wash, 5mins.



12. Incubate with secondary antibody.
13. x3 1XPBS wash, 5 mins.
14. Incubate in amplification step if necessary, followed by x3 1XPBS wash.
15. Incubate in DAB. (supersensitive and Impress kits)
16. Wash in dH₂O, 5 mins.
17. Wash in running dH₂O, 5 mins.
18. Incubate in Mayer's haematoxylin (~2 mins).
19. Wash in running tap water, 5 mins.
20. **In fumehood** - Dehydrate tissue through ethanol: 70%, 90%, 100%, 100%, 5 mins each.
Clear x 2 in Xylene, 5 mins and a final >30 min xylene.
21. **In fume hood** - Coverslip with DPX mountant (**reproductive toxicity hazard**) and leave to dry.

Nitrile dissolves after prolonged exposure to xylene so you **must** use the nitrile gloves as “splash protection only” – remove them immediately if they come into contact with xylene. You can wear xylene resistant gloves or use tweezers while working with xylene to reduce exposure time.

Accidental exposure/ first aid:

First Aid treatment for skin contact with xylene, ethanol, H₂O₂ and DPX:

Remove any contaminated clothing. Rinse skin well with water for a prolonged period. If necessary, seek medical attention.

First Aid treatment for inhalation:

Remove person to a well-ventilated area. If person seems to become dizzy or loses consciousness call security and wait for emergency services to arrive.

First Aid treatment for eye splash:

Rinse thoroughly for at least 15min using the eye wash station. If necessary, seek medical attention.

First Aid treatment for ingestion:

Do NOT induce vomiting. Rinse mouth with water and seek medical attention immediately.

First Aid treatment for scalds:

Flush the burn with lots of cool water for 20min. Seek medical attention if the patient has anything more than a small minor burn.

Emergency support through security: 4444 (+442075891000)

In all instances of accident OR near miss, notify the safety department and complete a SALUS report.

<https://www.imperial.ac.uk/safety/safety-by-topic/accidents--incidents/>

Occupational health contacts: <https://www.imperial.ac.uk/occupational-health/>

email: occhealth@imperial.ac.uk

phone: +44 20 7594 9401

Date	Name	Signature	Trained by	Supervisor/Lab Manager

