Standard operating procedure (SOP) for c-fos IMMUNOHISTOCHEMISTRY

All researchers performing a c-fos immunohistochemistry assay must be properly trained and supervised until they are deemed proficient.

Safety

- CAUTION: this assay requires the use of several moderately hazardous materials. A lab coat, gloves, facemask and safety goggles must be worn at all times.

DAB (3,3’-DIAMINOBENZIDINE; powder)

- Harmful - irritant. This product has the potential to cause adverse health effects with over exposure. Use safe work practices to avoid eye or skin contact and inhalation. When handled in small quantities the potential for dust inhalation and adverse health effects may be reduced. May cause cancer. Possible risk of irreversible effects.
- DAB has been listed as a HAZARDOUS SUBSTANCE according to the Safe Work Australia Criteria
- It is not classified as a dangerous good by the Criteria of the ADG code.
- This product presents a moderate hazard with normal use.
- Harmful to aquatic life. Do not let product enter drains. Discharge into the environment must be avoided.

XYLENE (clear liquid)

- Harmful - irritant. This product has the potential to cause adverse health effects with over exposure. Use safe work practices to avoid eye or skin contact and inhalation. Chronic exposure to some solvents may result in central nervous system (CNS), liver and kidney damage.
- Xylene is classified as a Schedule 6 (S6) Poison using the criteria in the Standard for the Uniform Scheduling of Drugs and Poisons (SUSDP).
- Xylene has been listed as a HAZARDOUS SUBSTANCE according to the Safe Work Australia Criteria and as a DANGEROUS GOOD by the Criteria of the ADG code.
- This product presents a moderate hazard with normal use.
- Harmful to aquatic life. Do not let product enter drains. Discharge into the environment must be avoided.
HYDROGEN PEROXIDE 30% w/v (clear liquid)

- Corrosive. This product has the potential to cause adverse health effects. Use safe work practices to avoid eye or skin contact and inhalation. Over exposure may result in severe and permanent eye, skin and respiratory damage. Upon dilution, the potential for corrosive effects may be reduced.
- Hydrogen peroxide is classified as a **Schedule 6 (S6) Poison** using the criteria in the Standard for the Uniform Scheduling of Drugs and Poisons (SUSDP).
- Hydrogen peroxide has been listed as a **HAZARDOUS SUBSTANCE according to the Safe Work Australia Criteria** and as a **DANGEROUS GOOD by the Criteria of the ADG code**.
- This product presents a moderate hazard with normal use.
- Harmful to aquatic life. Do not let product enter drains. Discharge into the environment must be avoided.

TRITON X-100 (clear liquid)

- Low to moderate toxicity - irritant. This product has the potential to cause adverse health effects with over exposure. Use safe work practices to avoid eye or skin contact and inhalation.
- Triton X-100 has been listed as a **HAZARDOUS SUBSTANCE according to the Safe Work Australia Criteria**
- It is not considered as a **DANGEROUS GOOD by the Criteria of the ADG code**.
- This product presents a moderate hazard with normal use.
- Harmful to aquatic life. Do not let product enter drains. Discharge into the environment must be avoided.

AMMONIUM CHLORIDE (white powder)

- Moderate toxicity - irritant. This product has the potential to cause adverse health effects with over exposure. Use safe work practices to avoid eye or skin contact and inhalation. Use safe work practices to avoid eye or skin contact and dust generation - inhalation.
- Ammonium chloride has been listed as a **HAZARDOUS SUBSTANCE according to the Safe Work Australia Criteria**
- It is not considered as a **DANGEROUS GOOD by the Criteria of the ADG code**.
- This product presents a moderate hazard with normal use.
- Harmful to aquatic life. Do not let product enter drains. Discharge into the environment must be avoided.
ETHANOL (clear liquid)

- Low to moderate toxicity - irritant. This product has the potential to cause adverse health effects with over exposure. Use safe work practices to avoid eye or skin contact and inhalation. Chronic exposure may result in cirrhosis of the liver. Over exposure may result in central nervous system (CNS) depression, with nausea, dizziness and unconsciousness at high levels.
- Ethanol is not considered a HAZARDOUS SUBSTANCE according to the Safe Work Australia Criteria
- It is considered as a DANGEROUS GOOD by the Criteria of the ADG code.
- This product presents a moderate hazard with normal use.
- Harmful to aquatic life. Do not let product enter drains. Discharge into the environment must be avoided.

MOUNTING MEDIUM (clear viscous fluid)

- Harmful - irritant. This product has the potential to cause adverse health effects with over exposure. Use safe work practices to avoid eye or skin contact and inhalation. Chronic exposure to some solvents may result in central nervous system (CNS), liver and kidney damage.
- Mounting medium is considered a HAZARDOUS SUBSTANCE according to the Safe Work Australia Criteria
- It is considered as a DANGEROUS GOOD by the Criteria of the ADG code.
- This product presents a moderate hazard with normal use.
- Harmful to aquatic life. Do not let product enter drains. Discharge into the environment must be avoided.

Procedure

A lab coat, gloves, facemask and safety goggles must be worn at all times.

Day 1

1. Incubate tissue for 30 min in 3% (30% w/v) hydrogen peroxide. To make 3% hydrogen peroxide add 3 mls of H2O2 for each 97 ml PB [phosphate buffer].
2. Rinse with phosphate buffered saline (PBS)
3. Incubate for 30 min in 3% normal horse serum
4. Rinse with PBS
5. Add primary antibody
   a. To make primary antibody:
      i. Make phosphate buffered horse serum (PBH):
      ii. Add 100 mg bovine serum albumin
      iii. 0.2 ml of Triton X-100
      iv. Add 2.0 ml normal horse serum
      v. Add PB to make 100 mls, stir well
      vi. Add 50 ul of primary antibody to 90 mls of PBH

6. Place vials on shaker for 30-60 minutes then refrigerate for 72 hours.
Day 2

A lab coat, gloves, facemask and safety goggles must be worn at all times.

1. Wash tissue with PBS for 30 minutes.
2. While you wait, make PBH as described in day 1.
3. Add 180 ul of secondary antibody
4. Place vials on shaker for 1 hour
5. Wash with PBS for 30 minutes
6. Add Extravidin and place on shaker for 2 hours
7. To make Extravidin:
   7.1 Add 90 ul to 90 mls of PBH
8. Wash 3x with PBS
9. CAUTION MUST BE TAKEN WHEN MAKING DAB- BE SURE YOU WEAR GLOVES, FACEMASK AND GOGGLES!!!
10. To make DAB: the following steps must be carried out in the FUME HOOD
   10.1 Weigh out 100 mg of DAB and add to 100 ml of PB.
   10.2 STIR for 10 minutes
   10.3 Add 2 mls of 0.4% ammonium chloride
   10.4 2 mls of D-glucose
   10.5 Add 2 mls of 2% nickel ammonium sulphate
   10.6 Stir until DAB is dissolved and the solution has a pink hue
   10.7 Filter through Whatman #1 paper into a graduated cylinder
   10.8 Top off with PB to make 200mls
11. Add DAB to vials and place on mixer for 20 minutes
   **ALL GLASSWARE AND INSTRUMENTS USED FOR DAB MUST BE WASHED WITH BLEACH**
12. Add glucose oxidase to each vial for 10 minutes
13. Decant DAB into a waste beaker- **DAB WASTE MUST BE PLACED IN A CLEARLY LABELED CHEMICAL WASTE CONTAINER WHEN YOU ARE FINISHED!**
   These containers are located on the left hand side under the bench top in surgery as you walk in.
14. Wash with PB for 30 min
15. Once finished place vials in refrigerator and be sure to mount tissue within a week.

Once tissue is mounted one must clear and cover them for permanent storage

Clearing

A lab coat, gloves, facemask and safety goggles must be worn at all times.

1. In the fume hood set up 6 glass wash boats (1 water, 1: 70% ethanol, 1: 95% ethanol, 1: 100 % ethanol, 2 xylene).
2. Carefully place mounted slides into a slide tray.
3. Dip the slide tray in the water boat for 30 seconds.
4. Then dip it into 70%, 95% and 100% ethanol for 1 min (each).
5. Repeat ethanol dips.
6. Place in first xylene boat for 5 minutes.
7. Then place in second xylene boat for 2 hours.
Coverslipping
1. Still in the fume hood, carefully removed slide from the xylene
2. Add mounting medium to the slide and cover with a glass coverslip
3. Using a cotton swab, carefully tap and press on the coverslip to remove air bubbles
4. Place on drying rack overnight
5. **IN THE FUME HOOD: XYLENE MUST BE PLACED IN A CLEARLY LABELED CHEMICAL WASTE CONTAINER WHEN YOU ARE FINISHED!** These containers are located on the left hand side under the bench top in surgery as you walk in.

Storage
1. Use xylene to remove any excess mounting medium before storing slides away in a proper slide box.
2. **IN THE FUME HOOD: XYLENE MUST BE PLACED IN A CLEARLY LABELED CHEMICAL WASTE CONTAINER WHEN YOU ARE FINISHED!** These containers are located on the left hand side under the bench top in surgery as you walk in.