Standard Operating Procedure (SOP) for Immunohistochemistry (IHC) Facility

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Facility name: Immunohistochemistry (IHC) Facility
Location: Room 204B, Department of Biosciences and Bioengineering (BSBE), IIT Bombay, Powai, Mumbai - 400076

Contact information:
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1. User registration:

- Users must **register for a time slot** before using the facility.
- During registration, users must **clearly specify the type and number of samples**.
- Users should fill out a **user form**

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2. Applications of histology and immunohistochemistry:

- **Understanding tissue structure:** Analysis at the cellular level to comprehend organ function.
- **Disease diagnosis:** Identifying pathological conditions such as cancer or infections.
- **Research and development:** Studying drug effects, disease mechanisms, and therapeutic interventions.

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3. Safety and hygiene protocol:

- Wear **lab coat/gown and gloves** at all times.
- Use **eye protection and face masks** when handling chemicals or tissues.
- Handle bulk tissues only in the **designated grossing area**.
- Identify **required area for sectioning** in bulk tissue during grossing.
- Dispose of **biohazard waste** in **yellow biohazard bags** as per safety guidelines.

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4. Protocol steps:

i. Fixation:

- Fix tissues in **4% PFA or 10% formalin** to preserve structure.

ii. Grossing:

- Perform **macroscopic examination**, dissection, and sampling.
- Select areas based on **organ type and clinical relevance**.
- Use **sterile blades and instruments**.
- **Document sampled regions** with labeled images or notes.

iii. Dehydration (STP 120 tissue processor):

- Wrap sections in **Whatman paper**.
- Load labeled cassettes into steel buckets.
- Place buckets in processor and run the following cycle:
    - 50% ethanol: 1 h
    - 60% ethanol: 1 h
    - 70% ethanol: 1 h
    - 80% ethanol: 1 h
    - 90% ethanol: 1 h
    - 95% ethanol: 1 h
    - 100% ethanol: 1 h
    - Xylene: 1 h (3x)
    - Wax: 1 h (2x)

iv. Embedding (Thermo Scientific):

- Preheat machine (60°C, 1 hour).
- Add molten wax to steel mold, place cassette, then cover with wax.
- Place mold on cold pad until wax solidifies.
- Demold and proceed for sectioning.

v. Microtome (HistoStar):

- Cut **5–10 µm sections**.
- Float sections in **40°C water bath**, mount on **charged slides**.
- Proceed to **H&E or immunostaining**.

vi. Cryostat:

- Maintain specimens at **-20°C to -30°C**.
- Pour **cryogel onto the stud**, allow to partially set.
- Cover with tissue and cryogel; wait **5–10 minutes** to solidify.
- Follow with **H&E or antibody-antigen staining**.

vii. Hematoxylin and eosin (H&E) staining:

- Stains **nucleus blue** (hematoxylin), **cytoplasm pink** (eosin).
- Used in **paraffin, frozen, or aspirate sections**.
- Performed **manually or via automated slide stainer**.

viii. Microscopic examination:

1. Mount stained slides.
2. Load into **fully automated computerized archival system**.

3. Set scanning mode (brightfield/fluorescence), resolution, magnification.
4. Initiate scanning using interface.
5. View and **verify image quality**.
6. **Annotate regions**, perform **image analysis**.
7. Save and **share results via the web-based database**.

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5. Fully automated computerized archival system:

- High-performance **automatic slide scanner**.
- Supports **brightfield and fluorescence** modes.
- Equipped with **dedicated cameras** and **user-friendly software**.
- **Web-based database** for image sharing, annotation, and storage.
- Suitable for **animal and human tissues**.
- Facilitates **collaborative analysis** by multiple users.

For queries, support, or feedback, please contact the facility operator listed above.