X-CLARITY TISSUE CLEARING SELF-SERVICE GUIDELINES
Mouse Histology & Phenotyping Laboratory

REAGENTS & CONSUMABLES

The following required supplies will not be provided by MHPL and must be procured by authorized users of the X-CLARITY System:

- 40% Acrylamide in Water (Cat#AAJ62480AP, Fisher Scientific)
- VA-044 Initiator (2,2'-Azobis[2-(2-imidazolin-2-yl)propane] dihydrochloride) (Cat# A301225G, Fisher Scientific)
- Supa Mega Tissue Cassettes (Cat # 22-222-023, Fisher Scientific) – *may be ordered as single pieces from MHPL*
- Macrosette Tissue Cassettes (Cat# NC0072902, Fisher Scientific) – *may be ordered as single pieces from MHPL*
- 20% Sodium Dodecyl Sulfate (Cat# BP1311-1, Fisher Scientific)
- 0.5 M Borate Buffer (pH 8.5) (Cat# NC1737708)
- 10x PBS buffer

IMPORTANT POLICIES

1. **Users must read the user manuals for X-CLARITY system which comprise of 3 units:**
    a. X-CLARITY polymerization system
    b. X-CLARITY Electrophoretic Clearing System
    c. Peristaltic Pump

    The instrument manuals and detailed X-CLARITY protocols can be found on the **MHPL Homepage**.
2. **Overview of X-CLARITY steps**
    a. Fixation: Specimens must be thoroughly fixed in 4% PFA at 4°C for the appropriate time. Intracardial systemic perfusion with 4% PFA followed by immersion fixation is highly recommended especially for CNS tissues (brain and spinal cord). The fixative should be at least 10x volume the tissue. Do not use neutral buffered formalin if you have endogenous fluorescent reporter proteins in the tissue to preserve their fluorescence properties.
    b. Rinse tissues in PBS.
    c. Equilibrate tissues in freshly diluted 4% Acrylamide/0.25% (w/v) VA-044 for 2 days at 4°C protected from light. The gel solution should be at least 10x the volume of the tissue.
    d. Polymerize the hydrogel in the X-CLARITY Polymerization System (37°C under vacuum for 3 h).
    e. Decant the excess polymerized hydrogel (must be collected in a hazardous liquid waste container and should not be disposed of in the sink).
    f. Perform electrophoretic clearing in 8% SDS/0.2 M Borate (pH 8.5; 2 litres, freshly prepared per run and should be stored at room temperature) using the following suggested settings:
        i. Current: 0.8-1.2 Amperes (lower amperage if temperature registers reach 40°C).
        ii. Temperature: 35-37°C
        iii. Peristaltic Pump: 30-60 rpm.
        iv. Time (variable): samples must be evaluated periodically to assess translucence.
    g. Rinse tissues several times in 1x PBS.
    h. Incubate tissues in Refractive Index Matching Solution (RIMS) – *may be ordered from MHPL*.
3. **Reagent Preparations**
    a. Required reagents and solutions must be prepared in the user's lab and not at MHPL.
    b. The VA-044 may be prepared as 100x stock in distilled or deionized water, aliquoted in small portions and stored frozen away from light.
    c. The 40% Acrylamide stock should be stored at 4°C.
    d. The clearing buffer (8% SDS/0.2 M Borate, pH 8.5) may be prepared in advance but must be stored at room temperature. Do not store in the fridge.

4. **Equipment reservation and operation**
    a. There are separate booking calendars for the X-CLARITY Polymerization and Electrophoretic Systems in **NUCORE**:
    b. **The X-CLARITY Polymerization has a fixed booking time of 3 h.**
    c. **The X-CLARITY Electrophoretic System can only be booked for the whole day (7 h window, 9:30 AM-4:30 PM on the booking calendar regardless of the actual start time. Each additional day must be booked separately.**
    d. Sign the logbooks for each instrument system and report any issues to MHPL staff ASAP.
    e. Users are responsible (not MHPL staff) for periodic checking of their electrophoretic clearing run:
        i. **Adjust amperage as needed to guarantee that the system does not overheat (≥40=°C).**
        ii. **It is best practice to lower the amperage to 0.8-1.0 A at the end of the day if continuing clearing overnight to avoid unexpected overheating.** The run may be resumed at higher amperage the following morning.
        iii. **Limit amperage to 1.2 A and below.** Overheating will damage the clearing chamber and destroy precious specimens.
        iv. Ensure that the clearing buffer (8% SDS/0.2 M Borate, pH8.5) is replenished each day if doing more than 1 day.
        v. Immerse the 2-L clearing buffer bottle in a water bath at ambient temperature to dissipate excess heat but never use an ice bath (SDS will precipitate at cold temperatures).
5. **Clean-up**
    a. Users are responsible for the disposal of liquid waste:
        i. **Excess polymerized hydrogel must be collected and disposed of as hazardous liquid waste. Do not dump into the sink!**
        ii. Spent tissue clearing buffer may be disposed in the nearby sink and flushed.
        iii. **The clearing buffer reservoir and electrophoretic clearing system must be rinsed thoroughly with deionized water.** Use the peristaltic pump (with the electrophoretic system TURNED OFF) to circulate clean water into the chamber and lines. Empty reservoir bottle completely after each run.
        iv. Tidy up the tissue clearing area after completing your run and take your own supplies to your lab.