# Clearing an adult mouse brain with the X-CLARITY™

The following is a *bis*-acrylamide and PFA-free protocol optimized for clearing an adult mouse brain with the X-CLARITY™ Tissue Clearing System. We recommend that you read the available online references on the CLARITY method and the X-CLARITY™ user manual before starting this procedure.

⚠ *Read the MSDS for all reagents. Wear the appropriate personal protective equipment. Work under a chemical fume hood when necessary.*

## Brain extraction, perfusion, and fixation

**Materials:** 4% PFA (cold)
1X PBS (cold)

1. Anesthetize the mouse ethically and responsibly.
2. Transcardially perfuse the mouse with at least 30 mL 1X PBS and 10 mL 4% PFA.
3. Rapidly decapitate the mouse and remove the brain.
4. Incubate the brain in 4% PFA for 24 hours at 4°C (do not exceed 24 hours).
5. Rinse the brain with 1X PBS several times.
6. (Optional) Wash in 1X PBS for 24 hours at 4°C.

**Note:** *The fixed brain may be stored in 1X PBS at 4°C for up to 3 months.*

## Hydrogel solution incubation

**Materials:** 40% Acrylamide (cold)
10% VA-044 (cold)
10X PBS (cold)
Distilled water (cold)

1. Mix up a fresh batch of the following in a 15 or 50 mL conical tube.

| | **Add** | **Final** |
|---|---|---|
| 40% Acrylamide | 1 mL | 4% |
| 10X PBS | 1 mL | 1X |
| 10% VA-044 | 250 µL | 0.25% |
| DDW | Up to 10 mL | - |

2. Incubate the brain in the mixture for 24 hours at 4°C (do not exceed 24 hours).

## Hydrogel embedding

High temperatures activate the thermal initiator VA-044 to begin free radical polymerization of the acrylamide monomers, which happens rapidly in the absence of oxygen. Biomolecules are covalently linked to the polyacrylamide network, forming a tissue-hydrogel hybrid. Upon completion, the brain will be a sticky, not quite jellified, substance.

**Materials:** Electrophoretic Tissue Clearing Solution (Cat # C13001)

1. Remove oxygen from the conical tube containing the brain and hydrogel solution in a desiccator.
2. Once the tube has been degassed, incubate for 3 hours at 37 °C (do not exceed 3 hours).
3. Rinse the brain with Electrophoretic Tissue Clearing Solution.

**Note:** *Do not store the brain in Electrophoretic Tissue Clearing Solution for an extended period of time as it will start being cleared passively. Proceed to the electrophoretic tissue clearing step as soon as possible.*

# Clearing an adult mouse brain with the X-CLARITY™

The following is a *bis*-acrylamide and PFA-free protocol optimized for clearing an adult mouse brain with the X-CLARITY™ Tissue Clearing System. We recommend that you read the available online references on the CLARITY method and the X-CLARITY™ user manual before starting this procedure.

*Read the MSDS for all reagents. Wear the appropriate personal protective equipment. Work under a chemical fume hood when necessary.*

## Electrophoretic tissue clearing with the X-CLARITY™

Read the X-CLARITY™ user manual carefully in its entirety prior to clearing the tissues. Use the system as specified.

**Materials:** Electrophoretic Tissue Clearing Solution (Cat # C13001)
1X PBS

1. Run with the following settings for 6-8 hours:

| | Recommended |
|---|---|
| Current | 1.5 A |
| Temperature | 37°C |
| Switching time | 0 min |
| Pump speed | 30 rpm |

**Note:** *Check the sample every 2 hours.*
**Note:** *If environmental temperature is high, lower the current to 1.2 A. This may increase clearing time.*
**Note:** *It is normal for the temperature to exceed the set temperature.*

2. Wash with 1X PBS O/N at RT to remove SDS.

**Note:** *The brain may be stored in PBS at 4°C for up to 1 week. The brain may become opaque during this time but transparency can be restored by incubating in X-CLARITY™ Mounting Solution.*

## Labeling/Imaging

**Materials:** X-CLARITY™ Mounting Solution (Cat # C13101)

1. Incubate with the appropriate antibodies.
2. Prior to imaging, incubate in an appropriate amount of X-CLARITY™ Mounting Solution for 1 hour at room temperature. Replace with fresh X-CLARITY™ Mounting Solution for an additional 1-2 hours.

   **Note:** *For optimal fluorescence imaging, do not leave samples in X-CLARITY™ Mounting Solution for more than 48 hours.*

3. Mount.
4. Image.