Animal Tissue Refraction Regulation Reagent

Introduction

Animal Tissue Refraction Regulation Reagent Solution II is a reagent designed to adjust the refractive index of tissues and organs. It can be used together with the Animal Tissue Optical Clearing Kit or independently for refractive index regulation of cleared tissue and organ samples.

Protocol

1. Tissue Sample Preparation

   a. Preparation of reagents and equipment

      a) Fixative: Use 4% paraformaldehyde fixative or immunostaining fixative.

      b) Anesthetic: Choose an appropriate anesthetic based on experimental needs, such as sodium pentobarbital or ketamine.

      c) Perfusion solution: 1X PBS/heparin sodium (10 U/mL) solution, or 1X PBS, or 4% paraformaldehyde fixative.

      d) Equipment: Surgical scissors, ophthalmic forceps, hemostatic forceps, infusion set needles (6 or 7 gauge for mice, 10–12 gauge for rats).

   b. Animal anesthesia: For example, anesthetize mice by intraperitoneal injection of 1% sodium pentobarbital (recommended dosage: 80 mL/kg body weight).

   c. Make a transverse incision below the ribs through the skin and abdominal wall, quickly cut the diaphragm, lift the sternum, and expose the heart.

   d. Insert the needle from the apex of the heart diagonally from the lower left to the upper right into the ascending aorta. If resistance is encountered, try changing direction; do not force the needle.

   e. Use hemostatic forceps to clamp the ventricle (or hold it by hand) to secure the needle and prevent leakage, then make a small cut in the right atrial appendage.

   f. Perfuse with 1X PBS/heparin sodium (10 U/mL). Start slow, then increase speed to flush blood vessels and remove blood. The standard for completion is clear fluid flowing from the right atrial appendage and the liver appearing grayish-white.

   g. Perfuse with 4% paraformaldehyde fixative for fixation. Start fast, then slow down until muscles harden.

The volume used is generally equivalent to the animal's body weight. Alternatively, dissect the required tissues or organs and then fix them.

h. Dissect the required tissues or organs and immerse them in 4% paraformaldehyde fixative. Fix overnight on a shaker at room temperature.

2. Preparation of Clearing Reagents

a. Preparation of 50% Solution I: Mix Solution I and distilled water in a 1:1 ratio. The mixture can be stored at room temperature for up to 1 month.

b. Preparation of 50% Solution II: Mix Solution II and 1X PBS in a 1:1 ratio. The mixture can be stored at room temperature for up to 2 weeks.

c. Preparation of 1X Wash Buffer: Dissolve 1 package of Wash Buffer in 2 L of water, then filter. The buffer can be stored at room temperature for several months.

3. Delipidation and Decolorization of Tissues and Organs

a. Discard the fixative, add 1X Wash Buffer, and wash on a shaker (60 rpm) at room temperature for 2 hours, repeated 3 times.

b. Add an appropriate amount of 50% Solution I to completely immerse the sample. Incubate on a shaker (60 rpm) at 37°C for 4–24 hours. The edges of the tissue will gradually become transparent.

***Note:** Adjust the volume of 50% Solution I based on the sample size to ensure complete immersion during shaking.

c. Add an appropriate amount of Solution I to completely immerse the sample. Incubate on a shaker (60 rpm) at 37°C, replacing the solution with fresh Solution I every 4 days until the tissue or organ is fully transparent.

***Note:** Adjust the volume of Solution I based on the sample size to ensure complete immersion during shaking.

d. Add an appropriate amount of 1X Wash Buffer to immerse the sample. Wash on a shaker (60 rpm) at room temperature at least 3 times, 2 hours each time.

4. Immunofluorescence Staining (Optional)

a. Primary antibody incubation: Dilute the primary antibody as recommended using an immunostaining primary antibody dilution buffer or other appropriate buffer. Incubate on a shaker (60 rpm) at 37°C for 3 days, protecting from light as needed.

Note: ***Note:** The dilution ratio can be adjusted based on actual staining results.

b. Recover the primary antibody, then wash with 1X Wash Buffer at room temperature 6 times, 2 hours each time.

c. Secondary antibody incubation: Dilute the secondary antibody as recommended using an immunofluorescence secondary antibody dilution buffer or other appropriate buffer. Incubate on a

shaker (60 rpm) at 37°C for 3 days, protecting from light.

Note: ***Note:** The dilution ratio can be adjusted based on actual staining results.

d. Recover the secondary antibody, then wash with 1X Wash Buffer at room temperature at least 3 times, 2 hours each time.

5. Nuclear Staining (Optional)

a. DAPI incubation: Dilute DAPI in 1X Wash Buffer to a working concentration of 0.5–10 μg/mL. Incubate on a shaker (60 rpm) at 37°C for 2–3 days, protecting from light.

***Note:** The dilution ratio can be adjusted based on actual staining results.

b. Wash with 1X Wash Buffer at room temperature at least 3 times, 2 hours each time.

6. Refractive Index Adjustment

a. a. Add an appropriate amount of 50% Solution II to immerse the sample. Place upright (horizontal placement may cause bubbles) and incubate on a shaker (60 rpm) at 37°C for 6–24 hours until the sample sinks.

***Note:** Adjust the volume of 50% Solution II based on the sample size; it is recommended to use at least 2–3 times the sample volume to ensure complete immersion during shaking.

b. Add an appropriate amount of Solution II to immerse the sample. Place upright and incubate on a shaker (60 rpm) at 37°C for 24 hours.

***Note:** The volume of 50% Solution I can be adjusted according to sample size, ensuring the sample remains fully immersed during shaking. Adjust the volume of Solution II based on the sample size; it is recommended to use at least 2–3 times the sample volume to ensure complete immersion during shaking.

c. Repeat step 6b once.

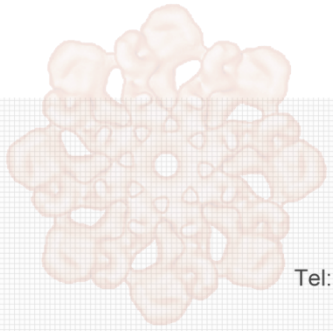
d. Thoroughly remove Solution II, add Mounting Solution to immerse the sample for 10 minutes, carefully remove bubbles from the sample surface, and then proceed with imaging.

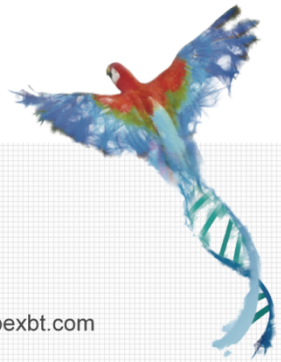
Note

1. Allow Solution II to thaw completely at room temperature before use. Ensure the solution is transparent and free of precipitate. If crystals or turbidity appear, briefly warm in a 55°C water bath until clear.
2. Solution II is inactivated at high temperatures (>75°C). Do not heat treat.
3. Do not shake Solution II vigorously to avoid bubble formation. If bubbles form, remove them using an ultrasonic cleaner, vacuum treatment (~0.1 MPa, ~30 min), or let the solution stand at room temperature (at least 7–8 hours) to allow bubbles to dissipate naturally. Otherwise, bubbles may appear around or inside tissues and organs.
4. Solution II is viscous. Use a wide-bore pipette tip (cut the tip of the tip with scissors) to slowly aspirate and

add the solution to the sample. Do not pipette vigorously to avoid generating bubbles.

5. After imaging and video capture, remove the Mounting Solution and store the tissue/organ samples in Solution II at room temperature for up to 1 week. Longer storage increases transparency but may also cause tissue swelling. After imaging, samples can be washed with 1X Wash Buffer, dehydrated in 30% (w/v) sucrose solution, embedded in O.C.T. compound, and stored long-term at -80°C.
6. Store Solution II at -20°C, protected from light, for long-term stability. For convenience, clearing can be performed without.
7. This product is for scientific use only.





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