### **Simplified Chemical Decellularization Protocol for Spinach Leaves**

#### **Materials and Reagents**

* Fresh spinach leaves
* Sodium dodecyl sulfate (SDS) solution (0.1% w/v)
* Triton X-100 solution (1% v/v)
* Phosphate-buffered saline (PBS)
* Distilled water
* Forceps and scissors
* Beakers or containers

#### **Procedure**

1. **Preparation of Spinach Leaves:**
   * Select fresh, healthy spinach leaves and remove any damaged or discolored parts.
   * Rinse the leaves thoroughly with distilled water to remove surface contaminants and debris.
2. **SDS Treatment:**
   * Prepare a 0.1% SDS solution in distilled water.
   * Submerge the spinach leaves in the SDS solution in a beaker or container. Ensure the leaves are fully immersed.
   * Gently stir or agitate the solution occasionally to ensure even exposure.
   * Allow the leaves to soak in the SDS solution for 24 hours.
3. **Rinsing:**
   * Remove the leaves from the SDS solution and rinse them thoroughly with distilled water.
   * Perform multiple washes (at least 3 times) to ensure all SDS is removed. Each rinse should last about 10-15 minutes with gentle stirring.
4. **Triton X-100 Treatment:**
   * Prepare a 1% Triton X-100 solution in PBS.
   * Submerge the rinsed leaves in the Triton X-100 solution.
   * Gently stir or agitate the solution occasionally.
   * Allow the leaves to soak in the Triton X-100 solution for 4-6 hours.
5. **Final Rinsing:**
   * After Triton X-100 treatment, remove the leaves and rinse them thoroughly with PBS.
   * Perform multiple washes (at least 3 times) to ensure all Triton X-100 is removed. Each rinse should last about 10-15 minutes with gentle stirring.
6. **Storage:**
   * Store the decellularized spinach leaves in PBS at 4°C until further use. Ensure the leaves are fully submerged to prevent drying out.