

## Standard Operating Procedure for Benzocaine Immersion Anesthesia for Frogs

**Supplies:** Benzocaine powder  
95% Ethanol  
100 ml flask or bottle with a tight fitting lid  
Scale  
100 ml cylinder or beaker  
Nitrile gloves  
Covered anesthesia container

### Preparation:

**This procedure is performed by the Investigator and the stock solution provided to FLSC.**

1. Wear Nitrile gloves when working with Benzocaine.
2. Weigh out 10 grams Benzocaine powder.
3. Measure 100 ml of 95% Ethanol and place into the bottle.
4. Add the Benzocaine powder to the Ethanol and dissolve.
5. Cap tightly and store at 4°C in the refrigerator.
6. Label as Benzocaine Stock Solution. Use the appropriate NFPA diamond label.
7. Record the date mixed and/or expiration date to the label.
8. Provide a current Safety Data Sheet to FLSC.

### Procedure:

1. Wear Nitrile gloves when working with Benzocaine.
2. Select an anesthesia container appropriate for the volume of immersion anesthesia to be used. The anesthesia container must have a lid as the animals will splash.
3. Use 1 ml of Stock solution in 1 liter of cold tap water for anesthesia of frogs.
4. Immerse frog in diluted Benzocaine solution to effect. Remove and assess depth of anesthesia by noting toe pinch response and breathing rate.
5. Diluted Benzocaine cannot be reused. Package for RM&S chemical pick-up and attach a Chemical Discard Tag to the outside of the container.