

# Visikol® TOX™ Alizarin Staining Technique:

## **GD18 Mouse Fetus**





### Introduction

This protocol is intended for use with fetal mice in developmental and reproductive toxicology (DART) studies. Visikol TOX greatly reduces the time required for staining skeletal systems for DART studies.

### **Protocol**

- 1. Eviscerate fetuses
- 2. Dehydrate in 95% ethanol (or isopropanol) for (at least) 24 hours.
- 3. Place into Visikol TOX Pre-Treatment Solution at room temperature for 60-120 minutes (25 mL per fetus).
- 4. After soaking in the Pre-Treatment Solution, briefly (~5 min) rinse the specimens in deionized water. Remove the specimens from the rinse water before proceeding with staining.
- 5. Prepare Alizarin Red S staining solution by dissolving 20 mg Alizarin in 500 mL of 1% KOH (Prepare fresh prior to use) for 24 hours (25 mL per fetus).
- 6. Remove from stain solution and transfer directly into Visikol TOX Post-stain solution for 2 hours (25 mL per fetus). Animals may be left in this solution indefinitely.
- 7. Specimens are transferred to Visikol TOX Clearing solution (25 mL per fetus) until clear (2-12 hours, may be left overnight)
- 8. After clearing, specimens may be stored in Visikol clearing solution indefinitely, or they may be transferred to aqueous glycerol for storage.

#### **Contact**

For any questions on the Visikol TOX process reach out to <a href="mailto:info@visikol.com">info@visikol.com</a>