

### CHDD room CD175: Anesthesia and Imaging of Transgenic Zebrafish SOP

Standard Operating Procedures for Zebrafish	
1. Chemicals/Hazards	Small volumes <1ml of embryo media, tissue culture media (DMEM), CSF, MS222 (zebrafish anesthesia)
2. Process	<i>[Describe or attach what is being done with Zebrafish, including specific laboratory procedures and quantities used.]</i> Fish are transported to CD175 in imaging dish. The dish is transported in a leak-proof secondary container such as a Styrofoam or plastic box. Fish are anesthetized with MS222 in the secondary container and moved onto the microscope platform for imaging. Fish are then transported back to CD056E in secondary container for euthanasia.
3. Preparation for use	<ul style="list-style-type: none"> <li>• Provide hazardous chemical and specific SOP training to personnel working with Zebrafish and any other personnel authorized or required to be in the laboratory or shared space during work with the agent.</li> <li>• Determine appropriate cleaning method(s) for Zebrafish. Ensure supplies for cleaning/decontamination are readily available.</li> <li>• Purchase or assemble supplies for a spill cleanup kit for Zebrafish. Ensure the kit is maintained, anticipated users are trained in its use and the kit is readily available in the laboratory.</li> </ul>
4. Environmental/Ventilation Controls	No special ventilation is required or present in the room.
5. Personal Protective Equipment (PPE)	<p><b>PPE</b></p> <p>The following PPE will be worn when working with Zebrafish:</p> <ul style="list-style-type: none"> <li>• Nitrile or latex gloves will be worn when handling fish containers.</li> <li>• Safety glasses are kept in a labeled drawer next to the microscope and worn when handling liquids.</li> <li>• Individuals will bring their own lab coat to CD175 and wear long pants or long skirt, and fully closed shoes.</li> </ul> <p>Gloves will be changed immediately if contaminated, torn, or punctured.</p>
6. Special Handling Procedures & Storage Requirements	<p><b>TRANSPORT</b></p> <ul style="list-style-type: none"> <li>• Zebrafish will be transported in labeled and covered non-breakable, leak-proof secondary containers.</li> <li>• Chemicals or reagents needed for imaging will also be transported to the room in non-breakable secondary containers.</li> </ul>
7. Spill and Accident Procedures <i>[Specific cleaning and waste disposal procedures must be determined.]</i>	<p><b>SPILLS</b></p> <ul style="list-style-type: none"> <li>• Potential chemical or liquid spill hazards that do not involve zebrafish would be of an aqueous, non-toxic nature and will be handled by the individual with paper towels and disposed in the municipal waste.</li> <li>• Potential spill hazards that involve zebrafish are treated as biohazardous waste. These spills will be handled by the individual with paper towels. Paper towels that have been used to clean up zebrafish spills will be placed in a biohazard bag and transported to the autoclave in CD046 for decontamination. A biohazard bag is stored in a labeled drawer next to the microscope. Individuals are responsible for replacing this bag if they use it.</li> </ul>

	For questions on spill cleanup, contact EH&S spill consultants at 206-543-0467.
<b>8. EXPOSURE PROCEDURES In Case of Emergency</b>	<p><b>EXPOSURE</b></p> <ul style="list-style-type: none"> <li>• A bottle of 60% Alcohol-based hand sanitizer is in CD175 in case of skin exposure.</li> <li>• After work is completed in CD175 and gloves are removed, <b>hand washing is required before exiting the room.</b></li> <li>• Since a hand-washing sink is not available in CD175, <b>hand sanitizer should be used instead.</b></li> </ul> <p><b>HAND SANITIZER</b></p> <ul style="list-style-type: none"> <li>• Hand sanitizer should always be used prior to exiting the room into the public hallway.</li> </ul> <p><b>NEAREST SINK AND EYEWASH LOCATION</b></p> <ul style="list-style-type: none"> <li>• <b>A hand washing station with eyewash is located in CD186H.</b> A map detailing the location of hand washing sink and an eyewash is posted on the door.</li> <li>• For all incidents and near misses, the involved person or supervisor completes and submits the <a href="#">UW Online Accident Reporting System (OARS)</a> form within 24 hours (8 hours if serious injury or hospitalization).</li> </ul>
9. Waste Disposal and Cleaning	<p><b>1. Waste Disposal:</b></p> <ul style="list-style-type: none"> <li>• <b>Chemical/liquid spills that do not involve zebrafish will be of an aqueous, non-toxic nature</b> and will be handled by the individual with paper towels and disposed in the municipal waste.</li> <li>• <b>Spills that involve zebrafish will be treated as biohazardous waste.</b> Paper towels that are used to clean up spills of this nature will be placed in a <b>biohazard bag</b> and transported to the autoclave room (CD046) in a covered, leak-proof secondary container.</li> <li>• Individuals will bring their own reagents and waste bottles in a leak-proof secondary container and transport everything back to CD056E for disposal.</li> </ul> <p><b>2. Cleaning:</b></p> <ul style="list-style-type: none"> <li>• Spills that <b>do not involve zebrafish</b> will be wiped up with paper towels and disposed in <b>municipal trash.</b></li> <li>• Spills that <b>involve zebrafish</b> will be wiped up with paper towels and disposed as <b>biohazardous waste</b> using a biohazard plastic bag.</li> <li>• No visible residues of any kind will be left on shared imaging equipment or work areas. See Block 12 for decontamination</li> </ul>
10. Special Precautions for Use of Zebrafish in Animals (if applicable)	Use of Zebrafish in animals will be documented and approved by IACUC. No special precautions are required.

Particularly hazardous substance involved?	<input type="checkbox"/> YES:	Blocks #11 to #13 are Mandatory
	<input checked="" type="checkbox"/> NO:	Blocks #11 to #13 are Optional.
11. Approval Required	All staff working with Zebrafish must be trained on this SOP prior to starting work. They must also review the Zebrafish SDS/MSDS, and it must be readily available in the laboratory. All training must be documented and maintained by the PI or their designee.	
12. Decontamination	All surfaces and non-disposable equipment will be decontaminated with 70% Ethanol.	
13. Designated Area	All work with Zebrafish must be done in a designated laboratory, work space CD056 and CD175.	
Name: Robin Gibson (PI or Lab Manager)		Title: Lab Manager
Signature:		Date: September 8, 2016

