Preparation of 80% Ethanol

1. SCOPE AND APPLICATION

This method details the preparation of an 80% ethanol solution, which is used in processing macroinvertebrate samples. See SOP IZ-02, IZ-03, IZ-04, IZ-06, IZ-07, IZ-13, IZ-39, and IZ-40.

2. SUMMARY OF METHOD

Ethanol (HPLC grade) (EtOH) and deionized (DI) water are mixed in the proper proportions, labeled, and stored.

3. APPARATUS AND EQUIPMENT

3.1 Graduated cylinder, 2000 mL
3.2 Glass amber container, 4 L
3.3 Plastic funnel to fill 4 L container
3.4 Safety equipment (see section 7.1)
3.5 Laboratory cart

4. REAGENTS AND CHEMICALS

4.1 Deionized (DI) water

4.2 Ethanol (HPLC grade) (EtOH) 100%

Note: Up to 60 L 100% EtOH (HPLC grade) may be stored in the flammable storage cabinet in the laboratory (B205). More can be found in Chemical Storage (C215).

4.2.1 Obtain a key to Chemical Storage (C215).

4.2.2 Take a cart and your safety equipment with you to the storage room. Before entering the room, have safety gear in place. Prop open the door.. Carefully place the EtOH container on the cart.

Note: If an accident or spill should occur, evacuate the room and immediately notify the Biology Safety Officer, Laboratory Safety Officer, Taxonomy Supervisor and Biology Program Administrator. If there is an injury, an alarm and wash down shower are outside the entrance to the chemical storage room. A washdown shower is also in the hallway outside of each lab.

4.2.3 Take the container to the laboratory (B205) and place it in the flammable storage cabinet.

4.2.4 Return the key to its proper location.

5. METHODS

5.1 Fill the graduated cylinder with 1600 mL of 100% ethanol.

Note: This operation is easier with two people as the ethanol is purchased in 20-L cans. One person holds the cylinder while the other pours the ethanol, if help is needed.

5.2 Add 400 mL of DI water to the graduated cylinder until the total volume is 2000 mL.

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5.3 Using the funnel, transfer the dilute ethanol into a properly labeled 4-L container as 80% ethanol. Repeat Steps 5.1 and 5.2 until final container is full.

5.4 Rinse the graduated cylinder and funnel with DI water and return both to the shelf above the sink.

5.5 Store remaining 100% EtOH and full containers of 80% EtOH in the flammable storage cabinet in room B205.

6. QUALITY CONTROL AND DOCUMENTATION

6.1 When a new container of 100% EtOH is opened, write the date opened and the analyst's initials on the label.

6.2 When a container of 100% EtOH is emptied, follow the pollution prevention and waste management step 8.1

6.3 Refer to the Laboratory Quality Manual.

7. SAFETY

7.1 Lab coat, latex or vinyl gloves, and eye protection are required when working with reagents and chemicals.

7.2 Avoid breathing Ethanol fumes. Keep face away from open containers of this chemical. Work in a well-ventilated area.

7.3 Refer to the MSDS for information about reagents and chemicals in this SOP.

7.4 Refer to the Laboratory Health and Safety Plan.

8. POLLUTION PREVENTION AND WASTE MANAGEMENT

8.1 Rinse empty EtOH containers three times with tap water, cross through the "flammable" label, mark the outside as "rinsed", and deposit in the trash container located in the parking lot.

8.2 Use caution when handling EtOH. Should an accident or spill occur, **immediately** notify the Biology Safety Officer, Laboratory Safety Officer, Taxonomy Supervisor and Biology Program Administrator. Proper clean-up and reporting procedures must be followed.

8.3 Refer to the Laboratory Health and Safety Plan.

9. REFERENCES

9.1 DEP Laboratory Quality Manual, https://floridadep.gov/dear/florida-dep-laboratory/content/dep-laboratory-quality-assurance-manual-and-sops

9.2 DEP Laboratory Health and Safety Plan, https://www.floridadep.net/resources/health-safety-manual

9.3 SOP IZ-02, *Stream Condition Index (SCI) Sample Preparation*

9.4 SOP IZ-03, *Invertebrate Hester-Dendy Sample Preparation*

9.5 SOP IZ-04, *Invertebrate Core/Grab/Dredge Sample Preparation*

9.6 SOP IZ-06, *Invertebrate Enumeration and Taxonomic Analysis*

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9.7 SOP IZ-07, *Subsampling of Worms and Midges*

9.8 SOP IZ-13, *Invertebrate Taxonomic Identification Quality Control*

9.9 SOP IZ-39, *Non-SCI Macroinvertebrate Sample Preparation*

9.10 SOP IZ-40, *Invertebrate Sorting Quality Control (QC)*

Appendix of Signifiicant Changes

01/19/2010	Added requirement to prop open the door of the Chemical Storage (C215).
01/13/2012	Added note to step 4.2.2 stating what to do if a spill occurs. Added step 8.3.
01/07/2014	Updated SOP # for Invertebrate Sorting QC.
12/29/2014	Formatted for 508 compliance.
01/07/2016	Updated name of the Quality Manual and links. Removed steps and references to the Ethanol Logbook.
02/28/2017	Standardized SOP naming conventions.
03/14/2018	Updated DEP Laboratory Quality Manual link.
02/09/2021	Updated link to the Health And Safety plan.
01/03/2024	Corrected minor grammatical errors.
12/10/2024	Changed volumetric unit from gallons to liters in Sections 4.2 and 5.1 for consistency
11/17/2025	Removed requirement of two people to retrieve 100% ethanol from chemical storage in section

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