

SOP for Microplastic Extraction from Clean Water Samples

PURPOSE

This SOP describes the procedure by which microplastics will be extracted from clean water samples. A laboratory blank should be run in addition to test samples, used to monitor particles introduced via procedural contamination.

OVERVIEW

Here, the sample will be analyzed with little sample preparation. The sample will be size fractionated to assist particle sorting by size.

MATERIALS

Item	Suggested Materials
Low foam dish soap	Alcojet detergent Fisher Catalog no. 16-000-111
Natural sponge	Amazon - "Natural Sea Sponge 6-7"
Aluminum foil	-
Laboratory Labeling tape	Fisher Catalog No. 15901A
Fine-tip sharpie	Sold at stationary stores
Squirt bottle (polypropylene)	Amazon – "Highfive 250cc Scientific Safety Wash Bottle Narrow Mouth Polypropylene/Plastic Squeeze Bottle Medical Label Tattoo Wash Bottle"
RO water	Alternatives include; MilliQ (18 MΩ cm), Deionized water or water filtered through a 1 μm pore-size filter
Filters	Material, pore size and diameter will vary based on study, sample type, analytical technique and filtering apparatus
Metal sieves	Mesh size and number of sieves will vary between studies
Metal sieve pan	Same diameter as sieves
Glass mason jars	>500 mL size One for each size fraction that will be wet picked Non-plastic lids preferred
Vacuum filtration system: 1 x Vacuum pump 2 x Plastic tubing 2 x 1000 mL Glass filtering flasks with rubber stopper 1 x filtering funnel 1 x filter holder with glass support 1 x metal clamp	GAST model DOA-P704-AA Tygon S3™ Laboratory Tubing Filtration set-up VWR Catalog no. 89428-970 Secondary filtering flask VWR Catalog no. 10545-858

Personal Protective Equipment (PPE)

The following PPE are mandatory for sample processing:

- Clean cotton lab coat
- Clean nitrile gloves
- Safety glasses, goggles or face shield when applicable (e.g. when working with reagents)
- Clean cabinet or covered enclosure to reduce contamination (if available)

PROCEDURE

Procedural Blanks

- One laboratory blank sample of clean RO water should be processed every 10 test samples, using the same protocol.

Preparation

- Before using any glassware or tools, wash with soap and water (surfactant helps to remove contaminant microplastics). Rinse three times with tap water, then three times with RO water (or suitable equivalent).
- Clean sieves with soap and water using a natural sponge.
- When equipment/tools/labware are not being used, or when samples are not being analyzed, keep covered to prevent procedural contamination.

Extraction procedure - Filtering

1. Set up sieve stack with largest mesh size on top, down to smallest mesh size on the bottom and sieve pan beneath (if counting to less than the smallest sieve mesh size).
 - a) Pour the sample through the sieve stack
 - b) Triple rinse the sample jar into the sieve stack using RO water. Tap the sieves gently to move everything through to its appropriate size fraction.
 - c) Rinse the contents of each sieve into a separate (cleaned and labelled) glass jar using RO water.

Alternatively, you can filter all size fractions onto filter paper. Either is acceptable, as long as you have them split into the relevant size classes dictated by the sieves (i.e. you can use wet or dry sorting for the larger size fractions).
 - d) Pour the contents of the sieve pan into a clean beaker and cover.
2. Assemble vacuum filtration system without the filtering funnel and clamp
 - a) Turn on vacuum pump. Pour RO water onto the glass filter holder to clean the system.
 - b) Turn vacuum pump off. Empty the waste from the bottom flask and rinse the flask with RO water, then reassemble.
 - c) Place your choice of filter onto the glass filter holder and secure the filtering funnel on top using the metal clamp.

3. Turn the vacuum pump on and pour the remaining sample (sieve pan contents, transferred to a beaker) through the filtration system.
Note: ensure not to overfill the filtering flask as this may lead to sample loss.
4. Keeping vacuum pump on, triple rinse the sides of the filtering funnel with RO water.
5. Turn off the vacuum pump, remove the metal clamp and carefully lift the filtering funnel away from the base.
Note: Tweezers may be used to ensure the filter is not removed with the filtering funnel as you do this.
6. Turn on the vacuum pump and carefully rinse the base of the filtering funnel onto the filter, using RO water.
7. Turn off the vacuum pump, remove the filter from the filtration system, place it into a clean, labelled petri dish and cover.
8. If filtering down to a smaller size fraction, pour and triple rinse the contents of the filtering flask into a clean beaker and cover, reassemble the filtration system using a filter with a smaller pore size and repeat steps 3-7.