OPTICAL BRIGHTENER FIELD GUIDE

Equipment Needed

- Organic, untreated cotton tampons
- Gloves
- 365 nm UV LED black light flashlight
- Whirl-Pak® black photosensitive bags
- Waste bottle
- Tweezers (optional)
- Fine tip sharpie
- DI water bottle

Modified Bottle Method

- Clear plastic bottle with slits (Figure 1)
- Scissors/scalpel
- Monofilament fishing line or strong rope
- Weighted kettlebell or similar object

Whirl-Pak® Bag Method

• Sample bucket or extension pole

Method 1: Modified Bottle

Record Field Observations and Comments on the Monitoring Form.

Sample Deployment

- 1. Label a black photosensitive Whirl-Pak® bag with the site ID, date, and time of deployment and retrieval (repeat for each site).
- 2. Using gloves, rinse the bottle and cap 2X with DI water and check for optical brightener contamination using a UV LED black light flashlight. Tie monofilament line or rope around the bottle neck, ensuring it's long enough for full submersion. Using gloves, rinse the scalpel with DI water and cut up to 5 evenly spaced 4–6-inch slits along the bottle to allow water flow while protecting the tampon (Figure 1).

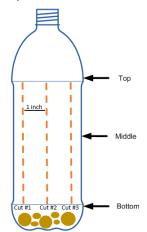


Figure 1. Modified Bottle

- 3. At the sampling site, use gloves to rinse the bottle and monofilament line 2X with sample water, discarding water downstream after each rinse.
- 4. Using gloves, unscrew the cap, carefully remove the tampon from its packaging without touching it, and hold it by the string. Place it halfway inside the bottle and secure the string by tightening the cap. Add pebbles if needed for weight (through the slits). Secure the bottle to a tree branch, rock, or weighted kettlebell via the monofilament line/rope.
- 5. Deploy the bottle at the site, ensuring it is fully submerged in the centroid of flow and shaded. Take a photo to document the location for retrieval and record the date and time of deployment on the Monitoring Form. If centroid deployment is not possible, see Method 2.

Sample Retrieval

- 1. Allow **24-72 hours** between deployment and retrieval of modified bottle. Record the date and time of bottle retrieval on the Monitoring Form.
- 2. Using gloves, retrieve the bottle and remove the tampon. If appropriate, quickly rinse with sample water to remove excess sediment. Place the tampon in the labeled Whirl-Pak® bag to prevent UV exposure until sample analysis.

Method 2: Whirl-Pak® Bag (for Coastal & Non-Accessible Sites)

Record Field Observations and Comments on the Monitoring Form.

Sample Collection

- 1. Before collecting the water sample, label each Whirl-Pak® bag with the site ID, date, and time collected.
- 2. Using gloves, collect a water sample. There are three ways to do this:
 - **Bucket Grab Method:** rinse bucket 2X with sample water and discard the water downstream. Grab the sample from the centroid of flow. Pour water from the bucket into the Whirl-Pak® bag never dip the bag into the bucket.

- Extension Pole Method: rinse pole 2X with ambient water downstream from the monitoring site. Attach the sealed Whirl-Pak® bag to the receiving carriage on the sampling pole using the yellow tabs. Immediately prior to obtaining the sample, remove the seal without touching the bag's interior. Open the bag with the tabs and invert it into the water, keeping the mouth downward to avoid introducing surface scum. Facing the bag upstream, tip it slightly upward to allow air to exit and the bag to fill. Avoid sediment contact.
- Whirl-Pak® Bag Directly: if the site is accessible, submerge the bag 0.3 m (1 ft), or roughly one-third of the depth, in very shallow streams. With the open end facing upstream, push the opened mouth of the bag upstream until full. Avoid sediment contact.
- 3. After collecting the water sample, using gloves, carefully remove the tampon from its packaging without touching it, and hold it by the string. Place the entire tampon in the Whirl-Pak® bag (Figure 2). Close the bag and invert 25X.

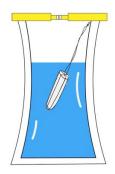


Figure 2. Whirl-Pak® Bag

4. Allow the tampon to soak in the Whirl-Pak® bag, positioned upright, in a dark environment for **24** hours.

Sample Analysis (Both Methods)

- 1. After the designated exposure period, in a dark setting with minimal light, use gloves to squeeze out as much water as possible. Place the tampon on a clean surface to prevent cross-contamination.
- 2. Gently unravel the tampon and expose as much

surface area as possible for analysis.

- 3. While exposing the tampon with the 365 nm UV LED black light flashlight, observe the tampon for distinctive blue fluorescence. Notes for Presence/Absence:
 - If distinctive blue spots are present within the cotton fibers of the tampon (gently pull apart fibers to confirm), even if small amount, mark as "Positive".
 - Blue flecks on the tampon surface are likely from contamination after retrieval. This does NOT count as a positive result. (When in doubt, use rinsed/sterilized tweezers to see if blue flecks are easily removed from surface).
 - Other colors may be visible with UV LED black light flashlight but do not reflect optical brighteners (red = photosynthetic material, purple = decaying organic material). A positive result refers ONLY to the blue fluorescence.
- 4. Document the presence and types of fluorescence, along with any comments on the Monitoring Form. If unsure about your markings or information, include your reasoning in the comments section and be consistent. Take photos of the exposed tampon and submit them with the Monitoring Form. It's best to use a dark background, hold the camera at the same distance as the flashlight, and, if possible, turn down your camera or iPhone exposure.
- 5. Dispose of the tampon in household waste. If using the modified bottle method, rinse the bottle, tweezers (if used), and scalpel/scissors 2X with DI water for reuse. As long as the bottle remains intact and is rinsed properly, it can be reused along with the monofilament line for the modified bottle method.

