

Faecal Source PCR Markers

1. Filter sample within 24 hours of collection. Water samples should not be frozen before filtering as this results in a reduction in sensitivity.
2. Filter up to 500 mL (minimum 100mls required) through 0.2µM Supor 200 filter using a vacuum manifold. If filters get blocked too quickly, a total of two filters / sample can be used – please record the total volume filtered.
3. Aseptically remove the filter(s) from the filter holder and place in a labelled **7mL tube**. Add 1ml of GITC buffer to the filter(s). When GITC buffer is added use the pipette tip (still attached to pipette) to fold/squash filter(s) so they are fully submerged in the buffer (at the bottom of the tube) and are thoroughly saturated. Secure lid firmly and vortex, then leave to settle 5min at room temperature. NB: If filter(s) are punctured by the filter tip they are still OK to process.
4. **Please seal tubes with parafilm** – it is very important that the GITC buffer does not leak from the tubes when they are transported to ESR.
5. Place the **7mL tubes** (upright in a rack) in -20°C freezer until all samples are ready to be sent to ESR. Filters can be stored for at least 6 months.
6. When all samples are ready for sending to ESR for analysis, place **7mL tubes** into a plastic bag, place bag into a chilly bin containing ice packs to keep the filters frozen, and send by overnight courier to the following address;
ESR Christchurch Science Centre
27 Creyke Road, Ilam
Christchurch
Attn: Molecular Biology Lab
7. Include a completed sample request form(s) and contact details for reporting.
Request form can be found at <http://www.waterquality.org.nz/>

Faecal Sterols Analysis

Must wear blue nitrile gloves while filtering samples to be analysed for sterols. This prevents contamination from sterols present on hands. Note nitrile gloves rather than latex ones must be used as latex polymers interfere with the analysis.

1. Filter up to 4 litres (L) of water through a GF/F glass microfiber filter. Generally for most water samples 2-4 litres is the typical volume filtered. If filters get blocked too quickly, a maximum of four filters / sample can be used – please record the total volume of water filtered.
2. Place filters into plastic container (either supplied by your own labs or one of the 50ml tubes provided) and clearly mark that the tube contains filters for **Sterol Analysis**.
3. Store frozen at -20°C until all samples are ready to be sent. Filters can be stored for up to 3 months.
4. When all samples are ready for sending to ESR for analysis, place them into a chilly bin containing ice packs to keep the filters frozen, and send by overnight courier to the address given above.
5. Include a completed sample request form(s) and contact details for reporting

Fluorescent Whitening Agents (FWAs)

At least 100 ml of water is required (200ml preferred). The sample should be protected from light so should be taken in an amber bottle or a clear bottle wrapped in kitchen foil and stored in dark at 4°C before shipping.

If required, samples can be frozen (stable for 6 months) while deciding whether to test or while collecting a group of samples over a period of time.

Once all samples have been collected / are ready for analysis they should be shipped by overnight courier in a chilli bin with ice packs to:

ESR Christchurch Science Centre
27 Creyke Road, Ilam
Christchurch
Attn: Molecular Biology Lab

Reagents and other Consumables

Please contact ESR at the email addresses below if you require:

- Sampling bottles for faecal source PCR markers, faecal sterols or FWAs
- 0.2µM Supor 200 filter for faecal source PCR markers
- 7mL tube for faecal source PCR markers
- GF/F glass microfiber filter for faecal sterols
- 50ml tubes for faecal sterols

faecalsource@esr.cri.nz or phone 03 351 6019 and ask for the molecular lab

Due to revisions in the rules from the Environmental Protection Agency and changes in the Hazardous Substances Regulations we can no longer supply the GITC buffer. The recipe is:

GITC Buffer (5M Guanidium thiocyanate (MW118.16), 100mM EDTA & 0.5% Sarcosyl)

To prepare 250mL:

Weigh out 147.7g Guanidine thiocyanate into a 300mL beaker.

Add 50mL 0.5M EDTA (pH8.0)

Add 1.25g Sarcosyl (N-laurylsarcosine)

Add approx. 50mL MilliQ dH₂O.

Place on a heated stirrer, add magnetic stirrer and apply low heat with stirring to dissolve.

Make up to final volume of 250mL in measuring cylinder.

Store at room temperature in a light proof container (amber bottle or wrap with foil).

Suppliers:

Guanidine Thiocyanate can be purchased from Lab Supply Cat# APPA1107,0250

0.5M EDTA can be purchased from Life Technologies cat# 15575020

Sarcosyl (N-Lauroylsarcosine sodium salt) can be purchased from Sigma #L9150-100G.