

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 the level of PCR markers present.
2. Filter up to 1000mL (minimum 200mls required) through 0.2µM Supor 200 filter using a vacuum manifold. If filters get blocked too quickly, a total of two filters per sample can be used. Note that filtering samples using 0.45µM filters is also likely to give a reduction in the level of PCR markers present.
3. Aseptically remove the filter(s) from the filter manifold and place in a labelled **7mL tube** – if used more than 1 filter for a sample then both filters must be placed in the same 7mL tube. These tubes can be supplied by ESR, see page 2
4. Record the total volume of water filtered and send this information to ESR along with the frozen samples. This information is required to calculate a quantitative result.
5. Add 1ml of GITC buffer to the filter(s) in the 7ml tube – if more than 1 filter still only add 1ml of GITC. 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. Recipe for GITC buffer is on page 2.
6. **Seal tubes with parafilm** – it is very important that the GITC buffer does not leak from the tubes when they are transported to ESR as any loss of GITC will reduce the level of PCR markers present.
7. 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 frozen for at least 6 months.
8. 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: Faecal Source Tracking
9. 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 filter gets blocked too quickly, a maximum of four filters per sample can be used.
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**. If multiple filters are used for the same samples, place all filters for that sample into one container.
3. Record the total volume of water filtered and send this information to ESR along with the frozen samples. This information is required to calculate the sterols results.

4. Store frozen at -20°C until all samples are ready to be sent. Filters can be stored for up to 3 months.
5. 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.
6. Include a completed sample request form(s) and contact details for reporting.

Reagents and other Consumables

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

- Sampling bottles for faecal source PCR markers and / or faecal sterols
- 0.2µM Supor 200 filters for faecal source PCR markers
- 7mL tubes for faecal source PCR markers
- GF/F glass microfiber filters 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.