



Test Principle

The Faecal Pathogen Detection Kit is intended for the detection of *Salmonella* spp., *E. coli* O157, *Shigella* spp. and *Campylobacter* spp., based on molecular biology methods. Genes that are idiosyncratic, respectively, for the genus *Salmonella*, *E. coli* O157, the genus *Shigella* and genus *Campylobacter*, and an internal positive control are amplified in a single reaction (multiplex PCR) and detected afterwards by sandwich hybridization and colour development. The special assay format enables a strong reduction of the standard protocol for DNA hybridizations and processing at ambient temperature (20-30 °C), thus providing a considerable saving of time and avoiding the usual cumbersome incubation of hybridizations in water baths or hybridization ovens. In contrast to agarose gel electrophoresis, where only the length of a DNA fragment can be used for identification and which requires handling of ethidium bromide, a strongly carcinogenic substance, hybridization has a higher degree of specificity due to the specific binding of complementary DNA sequences to the PCR products, and uses less hazardous chemicals.

BioEnteric Faecal Pathogen Detection Kit

Test Procedure

1.0 Enrichment of faecal samples

Inoculate 1.00 – 1.20 g of faecal specimen in BioEnteric Enrichment Broth.
Incubate overnight (\geq 16 hrs) at 37°C.

2.0 DNA extraction

The BioEnteric Extract Procedure

Fill sonicator with hot water to level mark in bath.
Degas the water in the sonicator, prior to DNA extraction procedure, to ensure efficient extraction.
To degas set the sonicator: Time 15 mins, temperature set to maximum, power 140%, and press 'Degas' and 'Start'

Decontaminate work area (Ambion DNA Zap – 9891G & 9892G), and wipe with DI water.

Thoroughly mix broth after removal from incubator.
Centrifuge enriched faecal sample for 1 minute at 100g.

Transfer 200 μ l of the supernatant enriched faecal sample into a BioEnteric Extract tube (which contains 2 phase separation system).

Mix by inversion approximately 20 times.

Place in rack, and transfer into the sonicator, and sonicate for 30 mins.

Sonicator Setting – Temperature 75 - 95°C, Power 140%.

3.0 PCR Amplification

Remove PCR strips from kit, and place on a PCR rack.
Remove the cap from the PCR tubes (good practice to number the caps to coincide with the tube number).

Pipette 14 μ l of Molecular Biology Grade Water (kit A) into each PCR tube (recommended to use fresh tip for each transfer steps).

From the sonicated BioEnteric tube, pipette 10 μ l of the lower phase into a PCR tube.

To ensure that an accurate volume is transferred pipette up and down 2 – 3 times, while the pipette tip is in the lower phase.

1 μ l of UNG may be added to each PCR tube to avoid contamination.

Run negative control with each batch of tests. This is performed by transferring 24 μ l of MBG water and 1 μ l of UNG (if required) into a PCR tube.

Optional: Run positive control. This is performed by transferring 20 μ l of MBG water & 5 μ l of Positive Control. Prior to use the Positive Control solutions should be vortexed and centrifuge briefly.

Prior to placing the PCR tubes into the thermocycler, replace the caps.

Tap all tubes to remove bubbles and dislodge any droplet's on inside walls of the tube.

Select 'BioEnteric' PCR programme, and press RUN. (The programme will run for ~ 2 hours)

After amplification, samples can be used for hybridization immediately or stored at -20 °C.

Note: After completion of PCR, samples can appear turbid. This does not influence the performance of the assay.

4.0 Hybridisation & Colour Development

4.1 Preparation of Solutions

Remove kits from fridge & freezer, and allow solutions to reach room temperature.

Fill 'Wash Bottle' with Deionised Water, and place the channel 3 tubing into the Wash Bottle

Fill 'Substrate Buffer' bottle with substrate buffer, 1ml per sample + 5ml for priming of instrument, and place 'channel 1' tube into the bottle

Fill 'Wash Solution' bottle with Wash Solution, 4ml per sample + 5ml for priming of instrument and place 'channel 2' tube into the bottle.

Prepare *Detection Solution*. This is prepared by pipetting – volume required per sample to be analysed

1 ml Substrate Buffer
10 μ l Substrate Solution 1
10 μ l Substrate Solution 2

Place detection solution onto the AutoProcessor, and place 'channel 4' tube into the bottle

Prepare *Hybridisation Solution*. This is prepared by pipetting – volume required per sample to be analysed

0.5 ml Hybridisation Buffer
10 μ l Probe Mix

The solution is prepared in a reagent reservoir, and mixed by manually agitating the reservoir.

This solution will be added manually to the AutoProcessor.
This solution should be prepared fresh prior to use.

4.2 Denaturing of PCR product

Remove PCR tubes from thermocycler

Add 25 μ l of Denaturation Solution to each PCR tube, and incubate at room temperature for 10 mins.

4.3 Processing of Strips

Using the forceps, remove strips from tube, and place to top end of individual channels in AutoProcessor tray

Transfer all the solution from the PCR tube into the bottom end of individual channels, ensure NOT to place solution onto the strip – as the pH will destroy the probes.

Add 500µl of 'Hybridisation Solution' to the top end of each channel, and manually agitate the tray to mix both solutions.

Place tray onto the AutoProcessor, using locator pins as guide, and secure with metal bar.

Ensure Waste Bottle is empty, and that all tubes are located at the bottom of the bottle solutions.

Turn on instrument & instrument will enter Main Menu
Select program BioEnteric and select Run

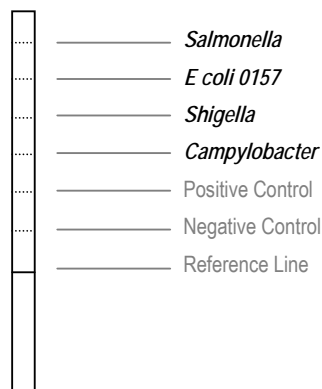
When program is complete, remove strips from tray and read
On program, select 'Liquid Prep', and 'Pump Back'.
The AutoProcessor will prompt 'Pump Back', select Yes

When this is complete all tubes should be placed in the water bottle, and start 'Auto Clean'

Kit	Contents	Storage Conditions
Part A	<ul style="list-style-type: none"> •Water, molecular biology grade, 2ml •PCR tubes, 24 units •De-naturation Solution, 15 ml •Probe Mix (colour code green), 400 µl •Wash Solution, sterile, 2 x 60 ml •Substrate Buffer, sterile, 65 ml •Test Strips 	+2...+8 °C
Part B	<ul style="list-style-type: none"> •Hybridization Solution, sterile, 20 ml •Substrate Solution 1 (purple cap), 350 µl •Substrate Solution 2 (white cap), 350 µl •Positive Control DNA (colour code colourless), 50 µl 	-20 °C

Equipment Required for Test
Incubator (37°C) Sonicator – Elma Transonic Digital S – TP 680DH. Centrifuge (suitable for 16mm diameter tubes) Pipette Filter Tips – Sterile 10 µl, 200 µl Pipette 2 x 10 µl, 2 x 1 ml, 2 x 200 µl, 2 x 50 µl Multichannel Pipette 300 µl, & 500 µl & Sterile tips Test Tube Rack PCR tube rack Thermocycler – Applied Biosystems 2720 Thermo Cycler BioEnteric AutoProcessor AutoProcessor Trays (48 channel)
Items not supplied with Kit
UNG – Uracil DNA Glycosylase BioEnteric Extract – DNA Extraction System BioEnteric Enrichment Broth
* supplied by Serosep Limited

Interpretation of Results



For illustration purposes only : not drawn to scale

A record sheet may be downloaded from www.bioenteric.com

Note:

The negative control line should not appear. If it appears the result is deemed invalid.

A result will be termed positive if any one of the lines is clearly visible (except the negative control).

The internal PCR control line is not required for a positive result.

