Fecal crude extract preparation for use with VersaTaq™ Direct PCR Polymerase
Product number 71002

Step 1: Starting material

For fecal sample stored in a stabilizer solution:
A. Centrifuge fecal sample at 3,000 RPM for 3 minutes and discard supernatant.
B. Wash fecal sample by gently resuspending in TE Buffer or PCR-Qualified Water and invert 6-8 times to facilitate washing.
C. Centrifuge at 1,000 RPM for 3 minutes and discard supernatant. Repeat wash step (b, c) x 2.
D. Resuspend fecal sample into appropriate volume of TE Buffer or PCR-Qualified Water.
Note: 50 mg feces in 1 ml solution.
E. Proceed to step 2.

For solid fecal sample:
A. Suspend 50 mg fecal material in 1 ml of TE Buffer or PCR-Qualified Water.
Note: Fecal suspension may be scaled based on amount of starting sample.
B. Proceed to step 2.

For liquid fecal sample:
A. Dilute liquid fecal sample 1:5 with TE Buffer or PCR-Qualified Water.
B. Proceed to step 2.

Step 2: Crude sample preparation

A. Homogenize fecal material until it is well suspended.
Note: If detecting microorganisms such as gram-positive bacteria or fungi, a sonication or bead beater step may lead to more efficient cell lyses.
B. Heat the suspension for 15 minutes at 70°C.
C. Quick spin at 1,000 RPM for 3 minutes, collect and save supernatant.
D. Add 1-5 µl of supernatant directly into the PCR reaction.
E. Follow recommended cycling conditions for ‘Crude extract from feces’ in the long VersaTaq protocol that accompanies the product (PN 71002).