

User Instruction for Total *Enterococcus* SmartBeads™

Extra care should be taken to prevent potential aerosol contamination.

Total *Enterococcus* SmartBeads

1. Total *Enterococcus* SmartBeads™: Scorpion Primer and Probe Set
2. Total *Enterococcus* SmartBeads™: Positive Control DNA

If the *Lactococcus* SPC Bead is used, first determine the amount of *Lactococcus* cells that need to be spiked into the water sample before filtration. A recommended amount is the amount that, after water sample prep, would result in Cy5 Ct between 25 and 32.

1. Total *Enterococcus* SmartBeads: Scorpion Primer and Probe Set

Reconstitute 1 “Total *Enterococcus* Scorpion Primer and Probe Set” Bead and 2 OmniMix HS Beads with 80 ul of molecular biology grade water. The reconstituted master mix is for 4 tests, with each test using 5ul sample to make up a total of 25ul PCR reaction.

The following PCR parameters are recommended with a total PCR time of about 50 min.

Target Dye	Dye Set Calibration	Cycling Parameters	Analysis Setting
FAM	FCTC25	95°C for 120 sec (1 cycle) For 45 cycles: 95°C for 5 sec 62°C for 43 sec (ON)	FAM threshold at 7, the rest as default.

2. Total *Enterococcus* SmartBeads: Positive Control DNA

Reconstitute 1 “Enterococcus DNA Bead” with 50 ul of molecular biology grade water, which results in reagent containing Enterococcus genomic DNA of 1000 CFUs/ul. Serial dilutions could be made from the reconstituted reagent to create a reference curve. The stated amount is for reference only, and is not intended to serve as a NIST-traceable standard. Individual investigator should make own correlation with existing method and revise accordingly. Use 5ul dilutions in a 25ul PCR reaction, with the master mix prepared in accordance with the procedure for “Total Enterococcus Scorpion Primer and Probe Set” beads.