

Materials List for:

A Duplex Digital PCR Assay for Simultaneous Quantification of the *Enterococcus spp.* and the Human Fecal-associated HF183 Marker in Waters

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Materials

Name	Company	Catalog Number	Comments
Low Bind Microtubes	Costar	3207	For storage of reagents, samples/production of master mixes
Nuclease Free Water	FisherSci	BP2484-50	
TE pH 8 buffer	FisherSci	BP2473-100	
Hardshell 96 Well Plate	BioRad	HSP-9601	For initial master mix and sample inoculation
Aluminum Sealing Film	BioRad	359-0133	To seal sample plate
Droplet Generator	BioRad	186-3002	
Droplet Generation Oil	BioRad	186-3005	
Cartridge	BioRad	186-4008	
DG8 Cartridge Holder	BioRad	186-3051	
Gasket	BioRad	186-3009	
20 µl pipet tips	Rainin	GP-L10F	For transferring sample/master mix to cartridge
200 µl pipet tips	Rainin	GP-L200F	For transferring droplets to final Twin.Tec Plate
Twin.Tec 96 Well Plate	Eppendorf	951020320	For final droplets thermal cycling and reading
Pierceable Heat Seal Foil	BioRad	181-4040	To seal Twin.Tec plate before thermalcycling
PX1 PCR Plate Sealer	BioRad	181-4000	
CFX96 Thermalcycler	BioRad	CFX96 and C1000	Only the thermal cycler is needed, no optics
QX100 Droplet Reader	BioRad	186-3001	
Droplet Reader Oil	BioRad	186-3004	
Droplet PCR Supermix	BioRad	186-3024	<i>i.e.</i> the digital PCR mix in manuscript
QuantaSoft software (v1.3.2)	QuantaSoft	QX100	For viewing, analyzing, and exporting ddPCR data
Entero Forward Primer (Ent F1A)	Operon	GAGAAATCCAAACGAACCTTG	Alternative vendor can be used
Entero Reverse Primer (Ent R1)	Operon	CAGTGCTCTACCTCCATCATT	Alternative vendor can be used
Entero Probe (GPL813TQ)	Operon	[6-FAM]-TGG TTC TCT CCG AAA TAG CTT TAG GGC TA-[BHQ1]	Alternative vendor can be used, but the fluorophore has to be FAM
HF183 Forward Primer (HF183-1)	Operon	ATCATGAGTTCACATGTCCG	Alternative vendor can be used
HF183 Reverse Primer (BthetR1)	Operon	CGTAGGAGTTTGGACCGTGT	Alternative vendor can be used
HF183 Probe (BthetP1)	Operon	[6-HEX]-CTGAGAGGAAGGTCCCC ACATTGGA-[BHQ1]	Alternative vendor can be used, but the fluorophore has to be HEX (if using VIC, then the appropriate

			<p>matrix compensation must be chosen.</p>
Positive control			<p>Mixture of <i>E. faecalis</i> genomic DNA and HF183 standard plasmid (ordered from IDT). For detailed methods in culturing <i>E. faecalis</i> and sequences of the ordered HF183 plasmid, please see Cao <i>et al.</i> 2015 (doi:10.1016/j.watres.2014.12.008). Commercially available <i>Enterococcus</i> DNA standards (ATCC 29212Q-FZ) can also be used in the positive control in place of lab-prepared <i>E. faecalis</i> genomic DNA.</p>