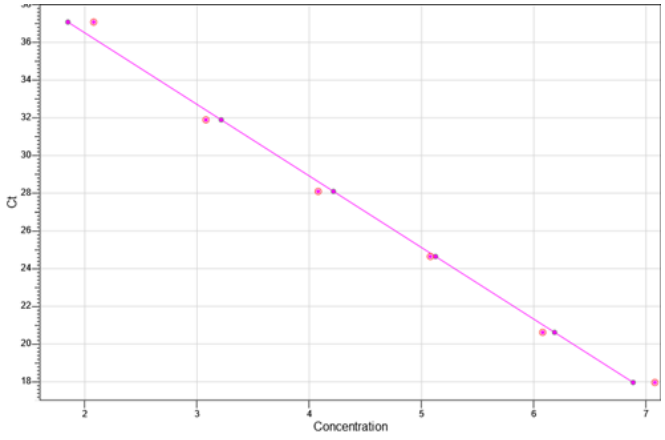
GeneCount™ qPCR Assay Datasheet

Assay Name: Sulfur-Oxidizing Bacteria
Catalog Number:

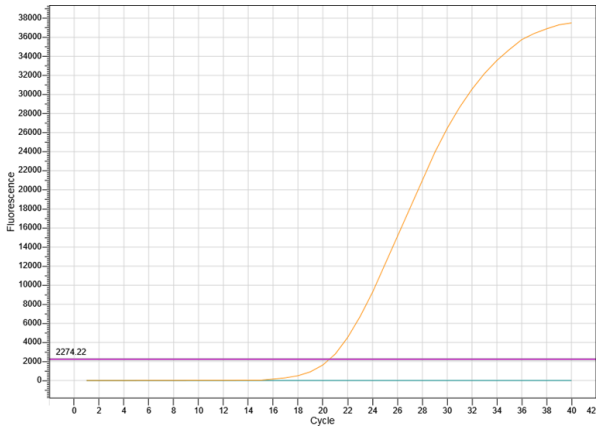
LUMINULTRA® microbial monitoring

Gene Information	
Assay Target	Bacteria that produce sulfite through oxidation
LOD (Gene Copies/ Reaction)	10^2
Assay Information	
Kit Components	GeneCount™ qPCR Master Mix Primer Mix Positive control DNA [2.4 x10^6 copies/RXN] Nuclease Free Water Resuspension Buffer 0.2 mL qPCR Tube Strips Aerosol Barrier Pipette Tips
Fluorescent Channels	SYBR
Cycling Conditions	95°C 3 Min - 1 Cycle 95°C 20s 60°C 45s] 40 Cycles

Standard Curve



Positive Control and Negative Control Amplification Plot



In vitro predicted partial coverage list	
Genus, species	**Strain number**
Allochromatium vinosum	DSM 180
Thioalkalivibrio versutus	-
Spirochaeta sp.	-
Marichromatium gracile	DSM 203
Thiocystis violacea	DSM 214, 198, 207
Ectothiorhodospira shaposhnikovii	DSM 243
Lamprocystis purpurea	DSM 4197
Halochromatium salexigens	DSM 4395
Rhabdochromatium marinum	DSM 5261
Sulfuriferula plumbiphila	DSM 6690
Thiobacillus denitrificans	DSM 12475
Thiobacillus thioparus	DSM 505T, NZ
Pandoraea sp.	ATSB25, ATSB26, ATSB27, ATSB30, ATSB32
Loktanella sp.	NP29
Thiomicrospira crunogena	HY-62
Paracoccus sp.	S6-2
Paracoccus denitrificans	-
Paracoccus versutus	DSM 582T
Rhodobacter sp.	BA15
Thiohalomonas denitrificans	HLD15
Marichromatium gracile	DSM 203
Thiocystis gelatinosa	DSM 215
Allochromatium minutissimum	DSM 1376
Rhodovulum adriaticum	DSM 2781
Ectothiorhodospira mobilis	DSM 4180
Thiorhodococcus minor	DSM 11518
Rhodobacter sp.	BA15
Thiohalorhabdus denitrificans	HLD10, HLD18

Assay Design

All GeneCount qPCR assay meet the following requirements.

1. Specificity

GeneCount qPCR assays are designed to target specific genes, each primer is designed to amplify this target and are meticulously selected using the most up to date reference databases. They are then screened both computationally and manually to ensure target detection with little off-target interference.

2. Coverage

During assay design, the genomes of thousands of different organisms are screened until each primer and probe* has the right combination of specificity and breadth of target recognition.

3. Compatibility

Although assays are designed around samples extracted using the GeneCount LT or HT purification kits, and the analysis features of the GeneCount Q-Series instruments, they are also designed to be compatible with many standard DNA purification methods and 4+ channel qPCR instruments.

4. Efficiency

Through careful optimization of assay reagents, reaction parameters, and calculations integrated with the GeneCount Q-Series software, assays must demonstrate limits of detection (LOD) and quantification (LOQ) within industry actionable limits, giving you results that matter.

5. Robust & Repeatable

Through extensive screening and analysis with real-world samples extracted from target sample matrices (Water, Wastewater, Fuel, etc.), GeneCount assays are capable of consistently detecting their target organism even within difficult sample types.

* Assay Dependant

Assay Validation

- All GeneCount assays have undergone extensive laboratory testing, wherein they were evaluated for all design criteria.
- All qPCR experiments were performed in duplicate, including (but not limited to) positive control, negative control, internal control (where applicable), NGS-confirmed environmental samples, and at minimum seven points from a tenfold dilution series of synthetic template (typically 2.4 million copies down to 2.4 copies).
- All assays were designed with proprietary mastermix reagents and were run in GeneCount Q-Series (Q-16, Q-48, and Q-96) devices.
- Data analyses were performed with GCQ-48 and GeneCount software with auto-integration and pre-set assay parameters.
- Amplification efficiencies and standard curves were calculated from the synthetic template dilution series or genomic DNA and NGS-confirmed environmental samples.
- Only assays that displayed linear performance from the LOD to a concentration of at least 10^6 GU/RXN range proceeded to further validation.
- Specificity was assessed by evaluating the length (bp) and melting temperature (Tm) of the amplified PCR product(s), wherein a single melt peak at a predicted Tm is expected for each primer combination within the assay.
- Sensitivity was determined through the assessment of non-specific amplification and primer-dimer formation, wherein assays with non-specific amplification detected in negative samples producing Ct values less than 35 were re-optimized or re-designed.