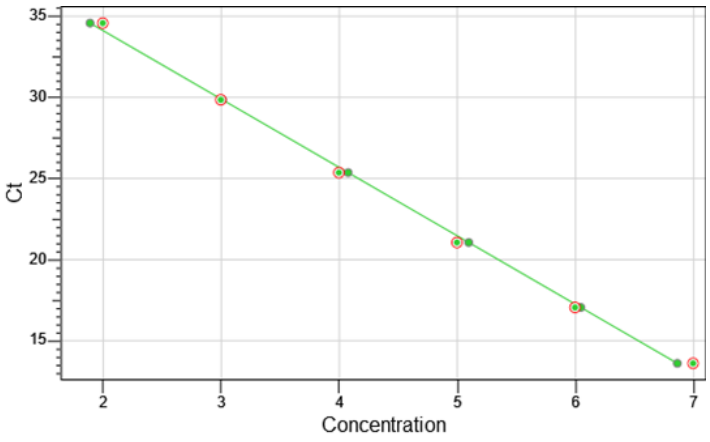
GeneCount™ qPCR Assay Datasheet

Assay Name: Methanogens
Catalog Number:

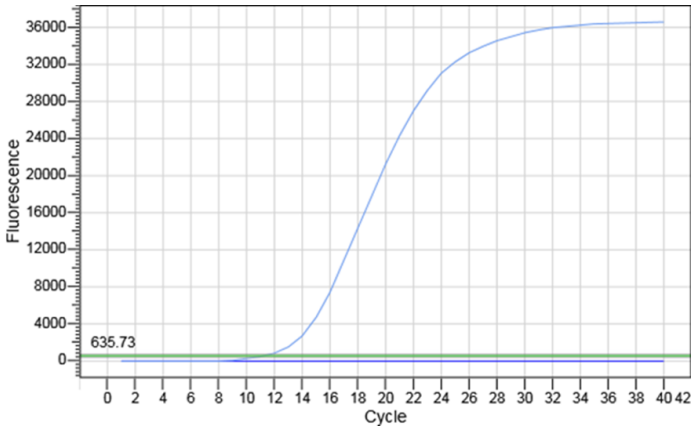
LUMINULTRA® microbial monitoring

Gene Information	
Assay Target	Microorganisms that produce methane gas
LOD (Gene Copies/ Reaction)	10^1
Assay Information	
Kit Components	GeneCount™ qPCR Master Mix Primer Mix Positive control DNA [1.98 x 10^6 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 partial coverage list	
Genus	**Species**
Methanobacterium	*formicicum, ivanovii, thermoautotrophicum, uliginosum, lacus, paludis, subterraneum, congolense*
Methanococcus	*vannielii, voltae, maripaludis*
Methanosaeta	*harundinacea*
Methanosarcina	*mazei, acetivorans, barkeri, flavescens, horonobensis, lacustris, siciliae, thermophila, vacuolata*
Methanothermobacter	*thermoautotrophicum, marburgensis, wolfeii*
Methanosphaera	*stadtmanae*
Thermoplasmatales	*archaeon*
Methanobrevibacter	*smithii*
Methanococcoides	*burtonii, methylutens*
Methanohalobium	*evestigatum*
Methanohalophilus	*halophilus, mahii, portucalensis*
Methanolacinia	*petrolearia*
Methanolobus	*psychrophilus*
Methanomethylovorans	*hollandica*
Methanosphaerula	*palustris*
Methanospirillum	*hungatei*
Methanotorris	*igneus*

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.