WESTON SOLUTIONS, INC.
5817 Dryden Pl, Suite 101
Carlsbad, CA 92008
(760) 795-6900 / (760) 931-1580
www.westonsolutions.com

# Weston Solutions qPCR Report - Sample Results

**Client:** County of San Diego
**Project:** SDR MST DW
**Survey:** Event 1 and 2
**Date Received:** 4/5 and 4/19/18
**n Samples:** 6
**Date Filtered:** 4/5 and 4/19/18
**Date Extracted:** 4/20/18
**Date PCR:** 4/23 and 4/26/18

**Assay:** HumanBacteroidales-HF183TaqmanCAMan
**µL Template per Reaction:** 5
**Lab Blanks:** passed n= 6
**No Template Controls:** passed n= 6
**Positive Extraction Controls[A]:** passed n= 6
**Inhibition Control[B]:** HF183 (B.dorei)
**File name(s):** Plate 285 HF183TMCaMan ka.pcrd
Plate 287 HF183TMCaMan ka.pcrd

| Sample ID | Site ID | Weston DNA ID | Date Sampled | Time Sampled | Matrix | Sample Result[C] | Qualifier[D] | Sample Concentration[E] | Sample Stdev[F] | Units[G] | SLOD[H] | SLLOQ[H] | cpr | Inhibition Result[I] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| MS4-SDR-098 | MS4-SDR-098 | 3690MS4-SDR-098 | 04/05/18 | 0906 | FW | Detected, ROQ | | 894 | 136 | copies/100mL | 29 | 58 | 92 | 0 |
| MS4-SDR-207 | MS4-SDR-207 | 3691MS4-SDR-207 | 04/05/18 | 0950 | FW | BDL | §< | 7 | 7 | copies/100mL | 29 | 57 | 1 | 0 |
| SDR-780 | SDR-780 | 3692SDR-780 | 04/05/18 | 1024 | FW | Detected, DNQ | | 35 | 7 | copies/100mL | 29 | 57 | 4 | 0 |
| MS4-SDR-207 | MS4-SDR-207 | 3703MS4-SDR-207 | 04/19/18 | 0935 | FW | ND | §< | 3 | 0 | copies/100mL | 29 | 57 | 0 | 0 |
| SDR-780 | SDR-780 | 3704SDR-780 | 04/19/18 | 1000 | FW | BDL | §< | 11 | 10 | copies/100mL | 29 | 57 | 1 | 0 |
| SDR-780 Dup | SDR-780 | 3705SDR-780 Dup | 04/19/18 | 1000 | FW | ND | §< | 3 | 0 | copies/100mL | 29 | 57 | 0 | 0 |

Abbreviations: Avg = Average; BDL = Below Detection Limit; cpr = copies per reaction; Cq = quantification (threshold) cycle; DNQ = Detectable But Not Quantifiable; FB = Field Blank; FW: Fresh Water; GW: Ground Water; L; SLT: Salt Water; SW: Storm Water; LOQ = Lower Limit of Quantification; LOD = Limit of Detection; n=number; N/A = Not Applicable; ND = Not Detected; NDsub = substitution value for nondetects; PCR = Polymerase chain reaction; rxs = reactions; StdDev = Standard Deviation; sub = substitution; TSC = Target Sequence Copies; ROQ = Range of Quantification; SLLOQ = Sample Specific Lower Limit of Quantification; SLOD = Sample Specific Limit of Detection.

Footnotes: [A]Sample Process Control (SPC), Sketa assay for salmon sperm. [B]Inhibition Control = assay used for 2 well spike with DNA dilution method. [C]Suggestion for conversion of sample result into categorical results: ROQ and DNQ = positive; ND = negative; BDL = equivocal (see explanation on Part B). [D]If shown: §Average computed for ND result by substituting Cq with maximum number of cycles (Boehm et al., 2013). [E]Concentration = mean of at least 3 technical replicates. [F]Standard Deviation of at least 3 technical replicates. [G]For enterococci, results are given in Target Sequence Copies (TSC), as per EPA Method 1611 (standard concs in TSC/ul = copies/ul x 4). [H]SLOD and SLLOQ: sample specific detection and quantification limits calculated based on sample specific processing volumes see more information on Part B. [I]Inhibition: 0 = no inhibition observed, 1 = inhibition observed, but overcome in diluted sample, 2 = inhibition not overcome in diluted sample: The given concentration may be underestimated for positive samples, 3 = Dilution needed to overcome inhibition did not yield amplification. Given concentration may be underestimated. NT = not tested. See Part B for additional comments.

WESTON SOLUTIONS, INC.
5817 Dryden Pl, Suite 101
Carlsbad, CA 92008
(760) 795-6900 / (760) 931-1580
www.westonsolutions.com

# Weston Solutions qPCR Report - Standard Curve Metrics

**Client:** County of San Diego **Project:** SDR MST DW **Survey:** Event 1 and 2 **Date Received:** 4/5 and 4/19/18

Comments:
For the sample listed below (Weston DNA-ID) the Inhibition control via B.dorei spike was not possible due to the HF183TMCaMan marker concentration (>20 cpr). Inhibition was assessed via internal process control (sketa) instead.
3690MS4-SDR-098

**Standard Curve Metrics***

| | | | |
|---|---|---|---|
| **Assay** | HumanBacteroidales-HF183TaqmanCAMan | | |
| % Efficiency | 99.32 | | |
| $r^2$ | 0.992 | | |
| slope | -3.34 | | |
| y-intercept | 38.52 | | |
| curve source | Weston Master Curve | | |
| standard source | genomic | | |
| **Site Conclusion Values** | **ND sub** | **LOD** | **LLOQ** |
| **cpr (copies per reaction)** | 0.4 | 3 | 6 |
| **Ct equivalent** | 40.00 | 36.93 | 35.93 |
| **LOD > % amplification** | 83 | | |
| **LLOQ StdDev** | 0.85 | | |
| Copies per genome | 7 | | |

* based on a master standard curve with a minimum of 50 data points.

AVG Filtration Volume 300 mL

Sample result calculations use cpr values based on the following definitions:
ND: Cq=maximum cycle number, negative result.
BDL: 0<Cq≤LOD, Equivocal result.
DNQ: LOD<Cq≤LLOQ, positive binary result.
ROQ: Cq>LLOQ, positive result.
LLOQ : lowest concentration with amplifcation rate of 100% (>20 reps).

In addition, SLOD and SLLOQ values are provided. These are sample specific detection limits which take into account sample processing, for example volumes or mass.

Categorical Results:
ROQ and DNQ = positive; ND = negative
BDL results are categorized as "equivocal" because concentrations below the LOD may not be definitively attributed to the source for which the assay was designed. Weston uses BDL concentration values to compute averages unless directed otherwise by Client. Sites with chronic BDL results may warrant additional monitoring.

Abbreviations: Avg = Average; BDL = Below Detection Limit; cpr = copies per reaction; Cq = quantification (threshold) cycle; DNQ = Detectable But Not Quantifiable; FB = Field Blank; LLOQ = Lower Limit of Quantification; LOD = Limit of Detection; n=number; N/A = Not Applicable; ND = Not Detected; NDsub = substitution value for nondetects; PCR = Polymerase chain reaction; rxs = reactions; StdDev = Standard Deviation; sub = substitution; TSC = Target Sequence Copies; ROQ = Range of Quantification; SLLOQ = Sample Specific Lower Limit of Quantification; SLOD = Sample Specific Limit of Detection.

Satomi Yonemasu Digitally signed by Satomi Yonemasu
Date: 2018.04.27 09:35:22 -07'00'

QC Officer (Satomi Yonemasu) Date