



Helbing Laboratory eDNA Technical Bulletin

All eDNA tools are validated through a rigorous multi-step evaluation protocol that includes tests of DNA target specificity and amplification sensitivity¹⁻³.

General eDNA Assay Information

Target Species: Western Toad (*Anaxyrus (Bufo) boreas*)
Species Code: am-ANBO

eDNA qPCR Tool: eANBOS
eDNA qPCR Format: TaqMan

Gene Target: MT-ND1
Published in:

eDNA Assay Sensitivity Test Summary using gBlocks™ Synthetic DNA

LOD 0.2 95% CI 0.2-0.4 Copies/Rxn LOQ 0.8 95% CI 0.6-1.4 Copies/Rxn LOB 0 hits/8
LOQ_{continuous} 4 Copies/Rxn

Binomial-Poisson model for 8 technical replicates determined using eLowQuant R code⁴. When the LOQ < LOD, use the LOD for the LOQ. Enzyme: Immolase

eDNA Assay Specificity Test Information

Each qPCR reaction in the specificity assay contained 10 picograms of voucher target gDNA (n=25 technical replicates)

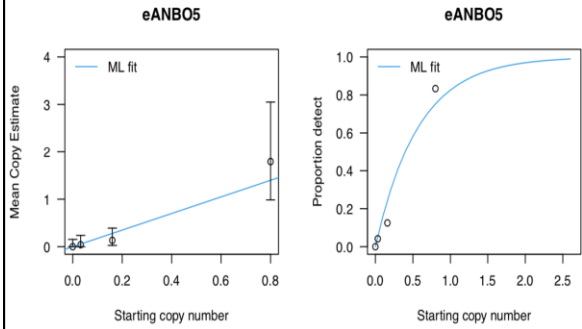
Species	Common Name (<i>Species</i>)	Detection	# Voucher	
			Specimens	Sample Sources/Locations
am-ANBO	Western Toad (<i>Anaxyrus (Bufo) boreas</i>)	Yes	7	British Columbia
am-ANVA	Wandering salamander (<i>Aneides vagrans</i>)	No	1	British Columbia
am-ASMO	Rocky Mountain Tailed Frog (<i>Ascaphus montanus</i>)	No	1	British Columbia
am-ASTR	Pacific Tailed frog (<i>Ascaphus truei</i>)	No	1	British Columbia
am-LICA	Bullfrog (<i>Lithobates (Rana) catesbeiana</i>)	No	1	British Columbia
am-LIPI	Northern Leopard Frog (<i>Lithobates (Rana) pipiens</i>)	No	1	British Columbia
am-LISY	Wood Frog (<i>Lithobates sylvaticus</i>)	No	1	British Columbia
am-PSRE	Pacific Chorus Frog (<i>Pseudacris (Hyla) regilla</i>)	No	1	British Columbia
am-RAAU	Northern Red-legged Frog (<i>Rana aurora</i>)	No	1	British Columbia
am-RACA	Cascades Frog (<i>Rana cascadae</i>)	No	1	British Columbia
am-RALU	Columbia spotted frog (<i>Rana luteiventris</i>)	No	1	British Columbia
am-RAPR	Oregon Spotted Frog (<i>Rana pretiosa</i>)	No	1	British Columbia
am-TAGR	Rough-skinned Newt (<i>Taricha granulosa</i>)	No	1	British Columbia
ma-HOSA	Human (<i>Homo sapiens</i>)	No	1	Netherlands

References

- Hobbs, J, Adams, IT, Round, JM, Goldberg, CS, Allison, MJ, Bergman, LC, Mirabzadeh, A, Allen, H, Helbing, CC (2020) Revising the range of Rocky Mountain tailed frog, *Ascaphus montanus*, in British Columbia, Canada, using environmental DNA methods. Environmental DNA. 2020; 2: 350-361. <https://doi.org/10.1002/edn3.82>
- Hobbs, J, Round, JM, Allison, MJ, Helbing, CC (2019) Expansion of the known distribution of the coastal tailed frog, *Ascaphus truei*, in British Columbia, Canada, using robust eDNA detection methods. PLOS ONE 14(3): e0213849. <https://doi.org/10.1371/journal.pone.0213849>
- Langlois, VS, Allison, MJ, Bergman, LC, To, TA, and Helbing, CC (2020) The need for robust qPCR-based eDNA detection assays in environmental monitoring and risk assessments. Environmental DNA, 3: 519-527. doi: 10.1002/edn3.164
- Lesperance, M, Allison, MJ, Bergman, LC, Hocking, MD, and Helbing, CC (2021) A statistical model for calibration and computation of detection and quantification limits for low copy number environmental DNA samples. Environmental DNA, 3: 970-981. doi: 10.1002/edn3.220



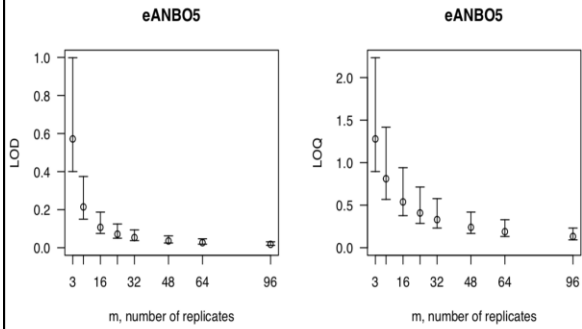
eDNA Assay Sensitivity Test Details using gBlocks™ synthetic DNA



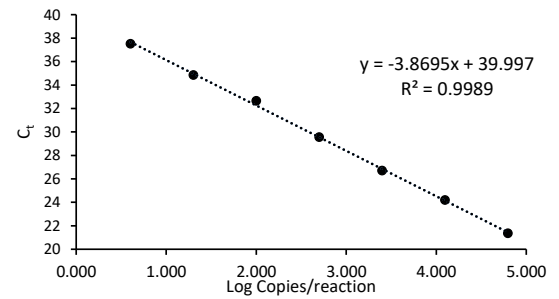
From 8 Technical Replicates

# Detects	# Copies	SE
0	0	0
1	0.076	0.078
2	0.17	0.12
3	0.27	0.17
4	0.4	0.22
5	0.56	0.29
6	0.79	0.39
7	1.19	0.6

Determined using eLowQuant R code⁴.



Applied to reactions with 100% positive hits



Binomial-Poisson model: No intercept

Determined using eLowQuant R code⁴.

Based on a 2 μ L DNA input in a total 15 μ L reaction

Efficiency 81%

Field Sample Validation

Sample Type	Known		Detected	Location
	Presence	# Samples		
Water	Y	8	Y	Southwestern British Columbia
Water	Y	15	Y	Northwestern British Columbia
Water	Y	12	Y	Yukon
Water	Y	10	Y	Northeastern British Columbia
Water	Y	2	Y	Nunavut
Water	Y	23	Y	Southeastern British Columbia

Abbreviations

95% CI	95% Confidence interval	LOQ	Limit of quantification
eDNA	Environmental DNA	MT-ND1	Mitochondrial NADH dehydrogenase 1 gene
gDNA	Total genomic DNA extracted from voucher specimen	NTC	qPCR no template control
LOB	Limit of blank	qPCR	Quantitative real-time polymerase chain reaction
LOD	Limit of detection	SE	Standard error