

APPENDIX S - Great Crested Newt EDNA Results

Pond 2 The Old Brickyard Farm South-West Pond

Client: Andrew Rothwell,



ADAS
Spring Lodge
172 Chester Road
Helsby
Warrington

Tel: 01159 516747
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Sample ID: ADAS-0870 Condition on Receipt: Low Sediment Volume: Passed
Client Identifier: 2 saw mill Description: pond water samples in preservative
Date of Receipt: 27/04/2021 Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control ¹	2 of 2	Real Time PCR	29/04/2021
Degradation Control ²	Within Limits	Real Time PCR	29/04/2021
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	29/04/2021
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 ⁻⁴ ng/μL) ³	4 of 4	Real Time PCR	As above for GCN

Report Prepared by: Dr Helen Rees Report Issued by: Dr Ben Maddison

Signed:



Signed:



Position: Director: Biotechnology Position: MD: Biotechnology

Date of preparation: 04/05/2021 Date of issue: 04/05/2021

eDNA analysis was carried out in accordance with the stipulated methodology found in the Technical Advice Note (WC1067 Appendix 5 Technical Advice Note) published by DEFRA and adopted by Natural England.

* If all PCR controls and extraction blanks give the expected results a sample is considered: negative for great crested newt if all of the replicates are negative; positive for great crested newt if one or more of the replicates are positive.

¹ Recorded as the number of positive replicate reactions at expected C_t value. If the expected C_t value is not achieved, the sample is considered inhibited and is diluted as per the technical advice note prior to amplification with great crested newt primer and probes.

² No degradation is expected within time frame of kit preparation, sample collection and analysis.

³ Additional positive controls (10⁻¹, 10⁻², 10⁻³ ng/μL) are also routinely run, results not shown here.

Pond 3 Old Dairy Farm

Client: Andrew Rothwell,



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Sample ID: ADAS-0869 Condition on Receipt: Low Sediment Volume: Passed
Client Identifier: 3 long copse Description: pond water samples in preservative
Date of Receipt: 27/04/2021 Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	2 of 2	Real Time PCR	30/04/2021
Degradation Control [‡]	Within Limits	Real Time PCR	30/04/2021
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	04/05/2021
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 ⁻⁴ ng/μL)*	4 of 4	Real Time PCR	As above for GCN

Report Prepared by: Dr Helen Rees Report Issued by: Dr Ben Maddison

Signed:



Signed:



Position: Director: Biotechnology Position: MD: Biotechnology

Date of preparation: 04/05/2021 Date of issue: 04/05/2021

eDNA analysis was carried out in accordance with the stipulated methodology found in the Technical Advice Note (WC1067 Appendix 5 Technical Advice Note) published by DEFRA and adopted by Natural England.

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[†] Recorded as the number of positive replicate reactions at expected C_t value. If the expected C_t value is not achieved, the sample is considered inhibited and is diluted as per the technical advice note prior to amplification with great crested newt primer and probes.

[‡] No degradation is expected within time frame of kit preparation, sample collection and analysis.

*Additional positive controls (10⁻¹, 10⁻², 10⁻³ ng/μL) are also routinely run, results not shown here.

Pond 4 Colman's Copse

Client: Andrew Rothwell



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Sample ID: ADAS-1511 Condition on Receipt: Low Sediment Volume: Passed
Client Identifier: 4 Colmans Description: pond water samples in preservative
Date of Receipt: 14/05/2021 Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control ¹	0 of 2	Real Time PCR	19/05/2021
Degradation Control ²	Within Limits	Real Time PCR	19/05/2021
Great Crested Newt ³	0 of 12 (GCN negative)	Real Time PCR	19/05/2021
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 ⁻⁴ ng/μL) ⁴	4 of 4	Real Time PCR	As above for GCN

Report Prepared by: Dr Helen Rees Report Issued by: Dr Ben Maddison

Signed:



Signed:



Position: Director: Biotechnology Position: MD: Biotechnology

Date of preparation: 20/05/2021 Date of issue: 20/05/2021

eDNA analysis was carried out in accordance with the stipulated methodology found in the Technical Advice Note (WC1067 Appendix 5 Technical Advice Note) published by DEFRA and adopted by Natural England.

¹ If all PCR controls and extraction blanks give the expected results a sample is considered: negative for great crested newt if all of the replicates are negative; positive for great crested newt if one or more of the replicates are positive.

² Recorded as the number of positive replicate reactions at expected C_t value. If the expected C_t value is not achieved, the sample is considered inhibited and is diluted as per the technical advice note prior to amplification with great crested newt primer and probes.

³ No degradation is expected within time frame of kit preparation, sample collection and analysis.

⁴ Additional positive controls (10⁻¹, 10⁻², 10⁻³ ng/μL) are also routinely run, results not shown here.