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
WA6 0AR

Tel: 01159 516747
Email: Helen.Rees@adas.co.uk

www.adas.uk

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[†]	2 of 2	Real Time PCR	29/04/2021
Degradation Control[§]	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)[#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by:	Dr Helen Rees	Report Issued by:	Dr Ben Maddison
Signed:	[redacted]	gned:	[redacted]
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.*

† 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 3 Old Dairy Farm

Client: Andrew Rothwell,

ADAS
Spring Lodge
172 Chester Road
Helsby
WA6 0AR

Tel: 01159 516747
Email: Helen.Rees@adas.co.uk

www.adas.uk

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:	[redacted]	Signed:	[redacted]
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.*

† 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

ADAS
Spring Lodge
172 Chester Road
Helsby
WA6 0AR

Tel: 01159 516747
Email: Helen.Rees@adas.co.uk

www.adas.uk

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[†]	0 of 2	Real Time PCR	19/05/2021
Degradation Control[‡]	Within Limits	Real Time PCR	19/05/2021
Great Crested Newt*	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 of 4	Real Time PCR	As above for GCN
Report Prepared by:	Dr Helen Rees	Report Issued by:	Dr Ben Maddison
Signed:	[redacted]	Signed:	[redacted]
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.

** 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.*

† 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.*