

eDNA SURVEY PROTOCOL

Kits should be kept at room temperature in an appropriate solvent store, consistent with Home Office regulations.

Kit contents: 1 sterile Whirl-Pak bag; 2 pairs of sterile gloves; 1 sterile sampling ladle; a sample box containing 6 x 50 mL sample tubes two thirds full of preserving fluid (contains alcohol); 1 sterile pipette; 1 protocol sheet.

Please **keep all packaging** as you will require this for couriered return of samples (see instructions emailed upon ordering and overleaf).

Don't go in the water.

- Collect your eDNA water sample before you do any other surveys at the pond.
- Take the sample whilst standing on the pond bank.
- Don't tread in the pond water itself either before or during collection of the DNA water sample as there is a considerable risk of contaminating your pond sample by bringing in Great Crested Newt DNA in mud and water from other areas on your boots and equipment.

Walk around the pond, to identify areas where you can take your eDNA samples

Roughly plan where you will collect the 20 water samples from. The aim is to spread the samples out evenly around the pond edge. The samples should be taken from both open water and vegetated areas if present and if possible should avoid water that is less than 10 cm deep. If you cannot access all areas of the pond, spread the samples out as best you can without entering the water. Existing data shows that eDNA can be patchy depending on where the animals have been. Sampling in many areas considerably increases the chance of collecting their eDNA successfully.

NOTE: Before you take each ladle sample, be sure to mix the pond the water column by gently using the ladle to stir the water from the surface to close to the pond bottom WITHOUT disturbing the mud in the bottom. DNA 'sinks' and so will often be present in larger amounts close to the pond bottom. **It is important not to collect sediment as this may cause inhibition of the PCR analysis which could lead to an inconclusive result** (please see examples of different sediment levels within sampling tubes at <http://www.adas.uk/Service/edna-analysis-for-great-crested-newt>).

SAMPLE COLLECTION

- Open your kit and put on a pair of gloves.
- Open the sterile Whirl-Pak bag by tearing off the clear plastic strip along the perforated line, then pull the tabs.

Collect 20 samples of 30 mL of pond water from around the pond (in the areas you have already identified) using the sampling ladle (fill the ladle), and empty each sample into the Whirl-Pak bag.

SAMPLE PRESERVATION

1. When you have collected your 20 samples, close the bag securely using the top tabs (fold over several times and bend tabs over) and shake the Whirl-Pak bag for 10 seconds. This mixes any DNA across the whole water sample.
2. Put on a fresh pair of gloves to keep the next stage as uncontaminated as possible.
3. Using the clear plastic pipette provided take 15 mL of water from the Whirl-Pak bag, and transfer into one of the six conical tubes containing preserving fluid (i.e. fill tube to the 50 mL mark).
4. **Label the box** containing the six tubes with the date, your name (sampler), the pond name, and grid reference/co-ordinates.

NOTE: Please do not overfill or under fill the tubes.

5. Close the tube and ensure the cap is tight - leaky samples could later contaminate the laboratory with DNA.
6. Shake the tube vigorously for 10 seconds to mix the sample and preservative.
7. Repeat for each of the 6 conical tubes in the kit.
8. Double check that the lids are on tightly if they have leaked during shaking please also wipe the tubes.
9. Empty the remaining water from the whirl-Pack bag back into the pond.
10. Place all used gloves, pipettes, rubbish into the sampling bag and dispose.

If storage of samples is necessary prior to their return please store refrigerated (2-4°C). Samples can be stored in this way for up to 1 month prior to analysis.

RETURNING THE KIT - DROP OFF OPTION

Should you wish to return your items directly to us, they can be dropped off at **Vet School Stores, SVMS, Nottingham University, Sutton Bonington Campus, Loughborough, LE12 5RD**. (please note opening times: 8.30am - 4.00pm Monday-Friday) or outside of these times at **Main Reception on College Road**. Please clearly mark your box "FAO Helen Rees: ADAS".

BOOKING YOUR DHL COLLECTION

Please email us at eDNAcouriering@adas.co.uk so we can arrange your collection.

We require the address of where the parcel will be, the number of parcels/number of kits, your contact details and the date of collection. Wherever possible we will try to book the requested date between 9am-5pm. Once we have booked your return we will email you the DHL collection documents, these will need to be printed off and attached to your parcel before your driver arrives. Please use original packaging wherever possible, if alternative packaging is used you **MUST** attach an **LQ label** (📄), we send along with your DHL collection documents just in case) and write **UN1170** onto the box or DHL will not transport your parcel. Should you have any problems please call the office on 01159 516747.