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Extract-N-Amp Equivalent DNA Extraction Protocol V.4

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Protocol status: Working

We use this protocol and it's working

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Abstract

This extraction protocol uses an Extract-N-Amp equivalent solution to quickly and cheaply perform DNA extractions. This protocol has been widely used for dried specimens of macrofungi, but will work for other organismal groups as well. This protocol is an amalgamation of two different protocols that are currently in use by academic labs in the United States.

This protocol is easily scalable for larger batch sizes. The standard protocol assumes 100mL batches. This is enough extraction solution for 5,000 specimens. The primary thing needed to scale up are larger sizes of the sterile vacuum filter flasks. They come in each size you may need - 250mL, 500mL, or 1000mL.

For larger batch considerations:

100mL is enough for ~5,000 extractions

250mL is enough for ~12,500 extractions

500mL is enough for ~25,000 extractions

1000mL is enough for ~50,000 extractions

Our lab typically make 500mL batches at a time.



Materials

Extraction Solution (ES) & Low TE Buffer

1 M Tris stock (pH 8.0-9.0) ([teknova](#)) 1000mL \$66.00 (enough for 50,000 specimens)

KCl ([Amazon](#) [Lab Grade or Reagent Grade]) 1 lb \$20.00

0.5 M EDTA ([IBI Sci](#)) 1L \$60.49

Molecular Water ([IBI Scientific](#)) 1L \$45.00

1 M NaOH ([Amazon](#)) 1L \$20.00

Equipment & Consumables

50mL Sterile Tubes ([Amazon](#)) - 50 tubes - \$20.00

250mL, 500mL or 1000mL Vacuum Flasks - Sterile 0.22um PES Disposable ([Ebay](#)) - \$50.00

Vacuum Pump ([Amazon](#)) - \$160.00

1000uL pipette tips

Scale

Up front equipment costs - \$440

~\$586 for first 50,000 extractions. \$0.0117/extraction

Ongoing costs are primarily the Tris stock and Vacuum flasks.

Create the Extraction Solution

- 1 In a flowhood or other clean environment, add  350 mL of molecular water. A sterile 50mL tube can be used for these measurements. We typically reuse empty molecular-grade water bottles as storage containers for this process.

For other batch sizes:

70mL for 100mL of end product in a 100mL flask.

175mL for 250mL of end product in a 250mL flask.

350mL for 500mL of end product in a 500mL flask.

700mL for 1000mL of end product in a 1000mL flask.

- 2 Add  50 mL of 1 M Tris stock pH 8.0-8.5 into a 100mL Erlenmeyer flask.

For other batch sizes:

10mL for 100mL of end product in a 100mL flask

25mL for 250mL of end product in a 250mL flask

50mL for 500mL of end product in a 500mL flask.

100mL for 1000mL of end product in a 1000mL flask.

- 3 Add  9.3 g of KCl. Swirl to dissolve.

For other batch sizes:

1.86g for 100mL of end product in a 100mL flask.

4.65g for 250mL of end product in a 250mL flask.

9.3g for 500mL of end product in a 500mL flask.

18.6g for 1000mL of end product in a 1000mL flask.

- 4 Add  10 mL of 0.5M EDTA.

For other batch sizes:

2mL for 100mL of end product in a 100mL flask.

5mL for 250mL of end product in a 250mL flask.

10mL for 500mL of end product in a 500mL flask.

20mL for 1000mL of end product in a 1000mL flask.

- 5 Add 1 M NaOH starting with  10 mL and then adding  1 mL at a time until the pH is above 9. The goal is to push just past the buffering range of Tris and end up with a pH between 9 and 9.5. This can be measured with pH paper or a pH meter. You do not want to add too much NaOH.



For reference, it takes us  13 mL of NaOH to push past pH 9 making 500mL of end product. For the products in the materials list, we now just add this amount without measuring pH each batch.

- 6 Top up your final solution to 100, 500, or 1000mL total volume (depending on how much you are making), using molecular grade water.
- 7 Filter sterilize your solution with the vacuum flask and pump. Put your final solution into sterile 50mL tubes. Store in 50mL tubes at  4 °C for up to 1 year.

Optional: Use low TE (10 mM tris + 0.1mM EDTA) to dilute extracts.

- 8 Add  1000 mL DNA grade 1 M Tris buffer pH 8.0-8.5 to a very clean 1 L vessel using a clean 50 mL tube to measure (typically just straight into the new Tris bottle once opened).
- 9 Add  2 mL 0.5 M EDTA using a pipette with sterile, filtered tips.
- 10 If prepared outside of a laminar flow cabinet or if using non-sterile reagents, filter sterilize using a 0.22 micron vacuum filter. Optional: Put your final solution into sterile 50mL tubes.

Use this solution to dilute your raw DNA extracts. A standard dilution is 1:10, however experiment with less or more to optimize your protocol.

Extraction Procedure

- 11 Put a small amount of tissue into one cell of an 8-strip tube. Maintain a spreadsheet of which specimens are going into which tube.
- 12 Add 20uL of Extraction Solution (ES) into each tube. For toothpick extractions we utilize 30uL of ES per tube.
- 13 Optional: Particularly for older specimens or recalcitrant tissue, add a 1.0mm stainless steel ball bearing to each tube, 50uL of low TE solution, and then vortex them for about 5 minutes. We have a BioSpec Mini-BeadBeater 96 and/or a Qiagen Tissue Lyser III for this purpose.



The addition of low TE at this point allows the tissue to be properly disrupted. With only 20uL of ES, the tissue tends to get stuck on the sidewall of the tube and not consistently fragmented.

Vortex the tubes for  00:00:05 and spin them down for  00:00:05 . We typically do this on a PCR rack and vortex 6 or 12 strips at a time, and spin down two plates at a time.

Note: We typically do not bead-beat most modern specimens that pass through for extraction - adding 80uL of low TE after the upcoming 10 minute incubation step - just before the template is about to be utilized into the final PCR plates. Bead-beating is typically not needed for 99% of specimens/species of macrofungi. For older fungarium/museum collections, or collections over 5 years old, the bead-beating process in this step (13) is critical. Success rates will significantly diminish without it.

- 14 Incubate the tubes for 10 minutes at  80 °C . This is typically performed in a thermocycler.

- 15 For modern specimens, we add 80uL of low TE solution at this point to further dilute the template after incubation. For toothpick extractions we utilize 40uL of low TE solution.

Note: If you added low TE with the previous bead-beating step, no more solution is added at this point.

- 16 Your extract is now ready to use 0.5 uL of the final solution as the template directly into a PCR reaction. Store at  -20 °C . DNA in this extraction solution can remain viable for years in the freezer.