VERSION 1

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Protocol status: Working
We use this protocol and it's working

Extract-N-Amp Equivalent DNA Extraction Protocol V.1

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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.

MATERIALS

Extraction Solution (ES)
1 M Tris stock (pH 8.0-9.0) (teknova) 1000mL $66.00
KCl (Amazon [Lab Grade]) 1 lb $20.00
EDTA (Amazon/IBI Sci) 100g $20.00
Molecular Water (IBI Scientific) 1L $45.00
1 M NaOH (Amazon) 1L $20.00

3% BSA Solution
BSA (GenDEPOT) - 50g $126.00

50mL Sterile Tubes (Amazon) - 50 tubes - $20.00
Titration Stand (Amazon) - $22.00
50mL Burette (Amazon) - $18.00
Phenolphthalein 1% Solution (Amazon) - $19.00
Vacuum Flask (Amazon) -
Vacuum Pump (Amazon) -

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Keywords: dna extraction,
extract-n-amp, fungi, mushrooms

Create the Extraction Solution

1 Add 10mL of 1 M Tris stock into a 100mL Erlenmeyer flask.

For larger batches:
50mL for 500mL of end product in a 500mL flask.
1000mL for 1000mL of end product in a 1000mL flask.

2 Add 1.86g of KCl.

For larger batches:
9.3g for 500mL of end product in a 500mL flask.
18.6g for 1000mL of end product in a 1000mL flask.

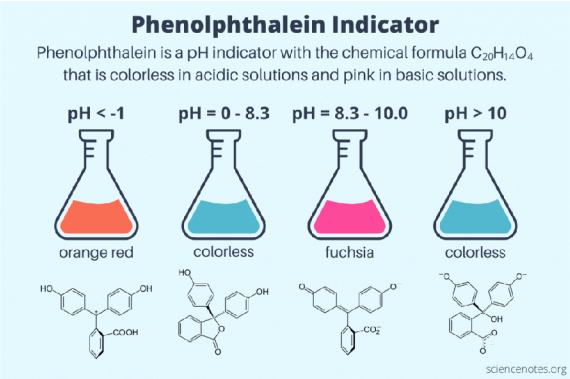
3 Add 0.37g of EDTA.

For larger batches:
1.85g for 500mL of end product in a 500mL flask.
3.70g for 1000mL of end product in a 1000mL flask.

4 Add 80mL of molecular water and shake until the solution dissolves.

For larger batches:
400mL for 500mL of end product in a 500mL flask.
800mL for 1000mL of end product in a 1000mL flask.

5 Add a two or three drops of Phenolphthalein to your solution. Setup your burette on the stand. Add 10 - 100 mL of 1 M NaOH to the burette (depending on how much you are making). Slowly release the NaOH into the flask until the solution turns from bright pink to colorless. This means you are now at a pH of 10.0.



Source

6 Top up your final solution to 100, 500, or 1000mL total volume (depending on how much you are making).

7 Filter sterilize your solution with the vaccum flask and pump. Put your final solution into sterile 50mL tubes.

Create the 3% BSA Solution

8 Add 3g of BSA into a clean 100mL flask.

For larger volumes:
Add 15g of BSA into a clean 500mL flask.
Add 30g of BSA into a clean 1000mL flask.

9 Top up to 100mL with molecular water.

For larger volumes top up to 500mL or 1000mL.

10 Shake the flask until the BSA is dissolved into the solution.

11 Filter sterilize the solution with a clean vacuum flask and pump. Put your final solution into sterile 50mL tubes.

Extraction Procedure

12 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.

13 Add 20uL of Extraction Solution (ES) into each tube.

14 Incubate the tubes for 10 minutes at 95C.

15 Add 20uL of 3% BSA solution to each tube.

16 Vortex the tubes for five seconds and spin them down for five seconds. 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.

17 Your extract is now ready to use 1uL of the final solution as the template directly into a PCR reaction.