Sep 09, 2025 Version 2

Modified Common Fungal DNA Extraction Buffer (XNA) V.2

Version 1 is forked from Extract-N-Amp Equivalent DNA Extraction Protocol

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

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Abstract

This is a modified version of an extraction buffer that is widely used for fungal DNA extraction. It works very well. The high pH and EDTA will protect DNA from nuclease activity, even at room temperature for long periods of time. It is inexpensive and uses common reagents to make. A short example method is provided. Here is another protocol that is used by the Fungal Diversity Survey. **dx.doi.org/10.17504/protocols.io.e6nvw1y59lmk/v2**

Materials

Extraction Solution (ES) and Dilution Solution (DS)
1 M Tris stock (pH 8.0-9.0) **Amaz**on 1000mL $25
KCl **Amazon** [Lab Grade] 1 lb $20.00
0.5M EDTA solution 500 mL **Amazon** 100mL **IBI Scientific** $30
Molecular-Grade Water **IBI Scientific** 1L $45 **Amazon** 3L $55
1 M NaOH **Amazon** 1L $20.00
pH paper **Amazon** - $25.00

Equipment & Consumables
50mL Sterile Tubes (**Amazon**) - 50 tubes - $20.00
250mL, 500mL or 1000mL Vacuum Flasks (**Ebay**) - $50.00 **Amazon** $60
Vacuum Pump (**Amazon**) - $160.00

Approximate up front costs:
Equipment costs - $240.00
Initial reagent costs - $125.00

~$360 for first ~50,000 extractions.

The majority of the volume of the extraction and dilution buffers are molecular-grade water, so buy in bulk if you want to make a lot of this buffer.

Create the Extraction Solution (500 mL batch)

1 In a laminar flow cabinet or other very clean space add approximately 350 mL of molecular-grade water into a very clean vessel capable of holding 500 mL with reasonably accurate volume gradations on the side. I reuse empty molecular-grade water bottles that have only been opened in a laminar flow cabinet for this purpose. You can probably use a glass flask or bottle if it is acid-washed and autoclaved.

2 Add 50 mL of 1 M Tris Buffer pH 8.0 I use a brand-new sterile, DNA free 50mL conical tube for this. You can re-use this tube to measure tris for other buffers.

3 Add 9.3 g of KCl (ACS or Reagent Grade) and dissolve by swirling the closed bottle.

4 Add 10 mL of 0.5 M EDTA.

5 Add 1 M NaOH starting with 10mL 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. One does not want to use too much NaOH and upset the electrolyte balance of the solution. While this is not precise, in practice it works very well.

6 Top up your final solution to 500 mL total volume using molecular grade water.

7 Filter sterilize using a DNAse free 0.22 micron vacuum filter. Alternatively, one can use syringe filters if a vacuum pump is not available, but this takes considerably more time and effort. 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 100 mL DNA grade 1 M Tris buffer pH 8.0 to a very clean 1 L vessel using a clean 50 mL tube to measure (you can use the one that you used previously for measuring tris). I typically buy a brand new bottle of of molecular grade water and decant 100 mL into 2 50 mL tubes and add directly to the bottle.

9 Add 200 µL 0.5 M EDTA using a p200 or p1000 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. Put your final solution into sterile 50mL tubes.

11 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.

Example DNA Extraction Method

12 Add 20 µL of extraction buffer to a standard PCR tube. Add a very small amount of tissue to the buffer, or add tissue first and buffer afterwards. 1mg is often too much for this volume of buffer. Allow to sit at room temperature for at least 10 minutes. Macerating or otherwise disrupting the tissue is optimal, but not always necessary. Heat PCR tube in a thermal cycler at 95 °C for 10 minutes. Dilute to preferred concentration and use right away, or store at -20 °C .