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Introduction

The E.Z.N.A.® HP Tissue DNA Maxi Kit is designed for efficient recovery of genomic DNA up to 60 kb in size from up to 2 gram of tissue samples. The special designed buffer system ensure the optimal lysis of tissue rich in fat, polysaccharides and fibers such as brain, adipose, muscles. This also can isolate DAN from molluscs, insects, arthropods, roundworms, flatworms, and other invertebrate tissue samples rich in mucopolysaccharides. The procedure relies on the well established properties of the cationic detergent, cetyltrimethyl ammonium bromide (CTAB), in conjunction with the selective DNA binding of Omega Bio-Tek's HiBind® matrix.

Samples are homogenized and lysed in a high salt buffer containing CTAB and digested with proteinase. After addition of chloroform, the homogenate is separate into aqueous and organic phases by centrifugation. The upper, aqueous phase is extracted and BL buffer and are added to provide appropriate binding conditions. The sample is then loaded into the HiBind® DNA Spin Column, where the genomic DNA binds to the membrane and salt and other contaminants are efficiently washed way. High quality genomic DNA is then eluted with Elution buffer or water. Purified DNA is suitable for most downstream applications such as endonuclease digestion, thermal cycle amplification, and hybridization techniques.

Storage and Stability

All components of the E.Z.N.A.® HP Tissue DNA Midi Kit are stable for at least 24 months from the date of purchase when stored at 22°C-25°C. During shipment or storage in cool ambient conditions, salts may precipitate in certain buffers. Simply warm to 37°C and mix to dissolve. Contents of the kit should not be refrigerated at any time.

Kit Contents

Product	D5196-00	D5196-01	D5196-03
Preps	2	10	25
HiBind® DNA Midi-Columns	2	10	25
15 ml Collecting tubes	2	10	25
Buffer MTL1	8 ml	20 ml	80 ml
Buffer BL	2 ml	10 ml	40 ml
Proteinase K	3 mg	30 mg	4 x 30 mg
RNase A	30 µl	270 µl	1.1 ml
DNA Wash Buffer Concentrate	12 ml	40 ml	3 x 40 ml
DNA Elution Buffer	1 ml	20 ml	50 ml
User Manual	1	1	1

Before Starting

- DNA Wash Buffer is supplied as a concentrate and must be diluted with absolute ethanol before first use. Add 18 ml *(D3473-00, 5 prep)*; 60 ml *(D3473-01, 50 prep)*; or 60 ml *(D3473-02, 200 prep)* ethanol to each bottle of DNA Wash Buffer Concentrate as indicated on the label. Once diluted with ethanol, DNA Wash Buffer must be stored at room temperature.
- Proteinase K - prepare a stock solution of 20 mg/ml water or TE and store in aliquots at -20°C

D3473-00 & D3373-00	Dilute with 150µl water or TE Buffer
D3473-01 & D3373-01	Dilute with 1.5ml water or TE Buffer
D3473-02 & D3373-02	Dilute with 1.5ml water or TE Buffer

- Buffer ML2 contains a chaotropic agent. Handle with care, always wearing disposable latex gloves and appropriate protective eye-ware.

Supplied By User

- centrifuge capable of 6,000 x g
- Sterile 15 ml microfuge tubes
- Absolute ethanol and 70% ethanol
- Water bath equilibrated to 60°C
- Sterile deionized water
- Chloroform - prepare Chloroform:isoamyl alcohol (24:1)
- Isopropanol (2-propanol)

HP Tissue DNA Maxiprep Protocol

samples preserved in formalin should be rinsed in xylene and then ethanol before processing. Note that results obtained with formalin-fixed tissues generally depend on age and size of specimen. Purified material is usually adequate for PCR amplification, but fresh or frozen samples should be used for southern analyses.

1. Pulverize 500 mg of tissue in liquid nitrogen with mortar and pestle and place the powder in a clean 15 centrifuge tube. Sample can also be ground and homogenized by beads mill.

Note: Amount of starting material depends on sample and can be increased if acceptable results are obtained with the suggested 500 mg tissue. In any event, use no more than 2 gram tissue per HiBind Maxi Spin-Column as DNA binding capacity (2.5 mg) may be exceeded. Meanwhile, difficult tissues may require starting with less than 500 mg tissue and doubling all volumes to ensure adequate lysis.

2. Add 9.0 ml Buffer MTL1 followed by 300 µl Proteinase K (20 mg/ml water). Vortex briefly to mix and incubate at 60°C for a minimum of 2 hours or until entire sample is solubilized. Actual incubation time varies and depends on elasticity of tissue. Most samples require no more than 4 hours. Alternatively an overnight incubation at 37°C will produce adequate results.

3. To the lysate add 9 ml chloroform:isoamyl alcohol (24:1) and vortex to mix. Centrifuge 4,000 x g for 5 min at room temperature. Carefully transfer the **upper** aqueous phase to a clean 15 ml microfuge tube. Avoid the milky interface containing contaminants and inhibitors. In most case, around 2 ml upper phase can be transferred.

Note: This step will remove much of the polysaccharides and proteins from solution and improve spin-column performance downstream.

4. **OPTIONAL:** Certain tissues such as liver have high levels of RNA which will be co-purified with DNA using this kit. While it will not interfere with PCR, the RNA may be removed at this point. Add 30µl (assuming a sample size of 500 mg) RNase A (25 mg/ml) and incubate at room temperature for 2 minutes. Proceed with the tissue protocol

5. **Add 0.5 x volume of Buffer BL and vortex to mix. Incubate at 70˚C for 10 minutes.** A wispy precipitate may form on addition of Buffer BL, but does not interfere with DNA recovery.
   Tip: For example if 2ml of aqueous phase is recovered in step 3, add 1ml Buffer BL.

6. Add 0.5 x vol absolute ethanol (96-100%) and mix throughly by vortexing.

   Tip: For example if the total sample volume is 3ml in step 3, add 1.5 ml ethanol.

7. Apply entire mixture, including any precipitation that may have formed, to an HiBind® DNA Maxi-column assembled in a 50 ml collecting tube (supplied). Centrifuge 4,000 x g for 5 min at room temperature. Discard flow-through liquid and collecting tube.

8. Place column into a clean 50 ml collecting tube and wash by adding 15 ml DNA Wash Buffer diluted with absolute ethanol. Centrifuge 4,000 x g for 5 min as above. Discard flow-through liquid and reuse collecting tube in next step.

   Note: DNA Wash Buffer is supplied as a concentrate and must be diluted with absolute ethanol as indicated on page 3 of this booklet.

9. Repeat step 8 with a second 15 ml DNA Wash Buffer. Discard liquid and using the empty collecting tube, centrifuge the column at 4,000 x g for 5 min at room temperature. This step is critical in removing traces of ethanol that will interfere with downstream applications (such as agarose gel electrophoresis of high molecular weight DNA).

9. Place column into a clean 50 ml centrifuge tube. To elute DNA add 3-4ml of DNA Elution Buffer (or 10 mM Tris buffer, pH 8.0) preheated to 60°C-70°C directly onto the HiBind matrix. Allow to soak for 2-5 min at room temperature. Centrifuge at 4,000 x g for 5 min to collect DNA.

10. Repeat elution step with a second aliquot of DNA Elution Buffer. Typically a total of 800 µg DNA with absorbance ratio (A_{260}/A_{280}) of 1.7-1.9 can be obtained from 1 gram animal tissue. Yields vary depending on source and quantity of starting material used.

    Note: Eluting DNA with 200 µl of Elution Buffer yield the same result as two elutions of 100 µl. To increase DNA concentration in final product, elute with 50 µl Elution Buffer. For high yields and concentration, elute once with 100 µl and repeat with the first eluate. Also, incubation of the column at 70°C following addition of Elution Buffer may increase yields.

Determination of DNA Quality and Quantity

Dilute a portion of the eluted material approximately 10-20 fold in DNA Elution Buffer or 10 mM Tris, pH 8.0. Measure absorbance at 280 nm and at 260 nm to determine the A_{260}/A_{280} ratio. Values of 1.7-1.9 generally indicate 85%-90% purity. The concentration of DNA eluted can be determined as follows:

Concentration = 50 µg/ml x Absorbance_{260} x {Dilution Factor}

Troubleshooting Guide

Problem	Possible Cause	Suggestions
Clogged Column	Incomplete lysis	Increase incubation time with Buffer MTL1 / Proteinase K. An overnight incubation may be necessary. Centrifuge to remove any insoluble particles.
	Sample too large	Do not use greater than recommended amount of starting material. For larger samples, divide into multiple tubes.
	Incomplete homogenization	Pulverize starting material as indicated in liquid nitrogen to obtain a fine powder.
Low DNA yield	Clogged column	See above
	Poor elution	Repeat elution or increase elution volume (see page for options). Incubation of column at 70°C for 5 min with dH_2O or Tris buffer prior to centrifugation may increase yields.
	Poor binding to column.	Follow protocol closely when adjusting binding conditions. adjust volumes of Buffer BL and ethanol in proportion.
	Improper washing	DNA Wash Buffer Concentrate must be diluted with absolute (100%) ethanol as specified on page 3 before use.
Low A_{260}/A_{280} ratio	Extended centrifugation during elution step.	Resin from the column may be present in eluate. Avoid centrifugation at speeds higher than specified. The material can be removed from the eluate by centrifugation — it will not interfere with PCR or restriction digests.
	Poor cell lysis.	Increase incubation time with Buffer MTL1 / Proteinase K. An overnight incubation may be necessary
	Trace protein contaminants remain.	Following step 8, wash column with a mixture of [300 µl Buffer BL + 300 µl ethanol] before proceeding to step 9.

Problem	Possible Cause	Suggestions
No DNA eluted	Poor cell lysis.	Increase incubation time with Buffer MTL1 / Proteinase K. An overnight incubation may be necessary.
	Incomplete homogenization	Pulverize starting material as indicated in liquid nitrogen to obtain a fine powder.
	Absolute ethanol not added before adding sample to column.	Before applying DNA sample to column, add Buffer BL and absolute ethanol as indicated in step 6, page 6.
	No ethanol added to DNA Wash Buffer Concentrate.	Dilute Wash Buffer with the indicated volume of absolute ethanol before first use.

References

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