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Introduction

The E.Z.N.A.® HP Tissue DNA Midi Kit is designed for efficient recovery of genomic DNA up to 60 kb in size from up to 500mg 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 DNA 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 buffer BL and are added to provide appropriate binding conditions. The sample is then loaded into the HiBind® DNA Midi 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, except the Proteinase K and RNase A can be stored at 22°C-25°C and are guaranteed for at least 24 months from the dated of purchase. Once reconstituted in water, Proteinase K must be stored at -20°C. Store RNase A at -20°C. Under cool ambient conditions, a precipitate may form in the Buffer BL. In case of such an event, heat the bottle at 37°C to dissolve. Store Buffer BL at room temperature.

Binding Capacity

Each HiBind® DNA Midi column can bind approximately 400 µg genomic DNA. Using greater than 500 mg tissue or 2 x 10^8 cells is not recommended.

Kit Contents

Product	D5197-00	D5197-01	D5197-03
Purification times	2 Preps	10 Preps	25 Preps
HiBind® DNA Midi Columns	2	10	25
15 ml Collection tubes	2	10	25
Buffer MTL1	8 ml	40 ml	80 ml
Buffer BL	8 ml	40 ml	70 ml
Buffer HB	7 ml	35 ml	80 ml
Proteinase K	3 mg	30 mg	2 x 30 mg
RNase A	50 µl	220 µl	550 µll
DNA Wash Buffer Concentrate	5 ml	20 ml	2 x20 ml
Elution Buffer	5 ml	30 ml	60 ml
User Manual	1	1	1

Materials to be Provided by User

- Laboratory centrifuge equipped with **swinging-bucket** rotor capable of 2000-5000 × g.
- Sterile 15 ml microfuge tubes
- Absolute ethanol (96-100%)
- Water bath equilibrated to 60°C
- Sterile deionized water
- Chloroform - prepare Chloroform:isoamyl alcohol (24:1)

Before Starting

- Please read the entire booklet to become familiar with the E.Z.N.A.™ HP Tissue DNA Midi Kit protocol.
- Dilute DNA Wash Buffer Concentrate with absolute ethanol as follows and store at room temperature.

D5197-00	Add 20 ml absolute (96%-100%) ethanol to bottle.
D5197-01	Add 80 ml (96%-100%) ethanol to bottle.
D5197-03	Add 80 ml (96%-100%) ethanol to each bottle.

- Prepare proteinase K stock solution as following:

D5197-00	Add 200µl Elution Buffer to the vial
D5197-01	Add 1.5 ml Elution Buffer to the vial
D5197-03	Add 1.5 ml Elution Buffer to each vial

Vortex vial briefly prior to use. We recommend that you aliquot and store vials of reconstituted protease at -20°C

Buffer BL contains a chaotropic salt. Use gloves and protective eyeware when handling this solution.

HP Tissue DNA Midi 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 200 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.

   Amount of starting material depends on sample and can be increased if acceptable results are obtained with the suggested 200 mg tissue. In any event, use no more than 500 mg tissue per HiBind™ DNA Midi Column as DNA binding capacity (400 µg) may be exceeded.Meanwhile, difficult tissues may require starting with less than 200 mg tissue and doubling all volumes to ensure adequate lysis.

2. **Add 3.0 ml Buffer MTL1 followed by 100 µl Proteinase K.** 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 55°C will produce adequate results.

3. **To the lysate add 3 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. If very few the upper aqueous presented, add 1ml of Buffer MTL1 into the lysate and vortex to mix again. Centrifuge as above, then transfer the supernatant into a new 15 ml tube.

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 50µl (assuming a sample size of 100 mg) RNase A (100 mg/ml) and incubate at room temperature for 30-60 minutes. Proceed with the tissue protocol.

5. **Add equal 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.

6. **Add equal volume of absolute ethanol (room temperature, 96-100%) and**

mix throughly by vortexing at maxi speed for 30 sec. If precipitation can be seen at this point, break the precipitation by passing through a needle using a syringe.

Tip: For example if the **total upper aqueous phase volume** is 2ml in step 3, add 2 ml Buffer BL and add 2 ml absolute ethanol.

7. **Apply the mixture from step 6, including any precipitation that may have formed, to an HiBind® DNA Midi-column assembled in a 15 ml collection tube (supplied)**. Centrifuge 4,000 x g for 5 min at room temperature. Discard flow-through liquid and repeat to apply the remaing mixture to the column, centrifuge as above to pass through the mixture. Discard flow-through and reuse the collection tube.

8. **Place column back into the 15 ml collection tube and wash by adding 3.0 ml Buffer HB**. Centrifuge 4,000 x g 2 min. Discard flow-through liquid and reuse collecting tube in next step.

9. **Place column back into the 15 ml collection tube and wash by adding 3.0 ml DNA Wash Buffer diluted with ethanol**. Centrifuge 4,000 x g 2 min. 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 4 of this booklet.*

10. **Repeat step 9 with a second 3.0 ml DNA Wash Buffer. Discard liquid and using the empty collection tube, centrifuge the empty column at maxi speed (no more than 8,000 x g) for 10 min at room temperature**. **This step is critical in removing traces of ethanol that will interfere with downstream applications.**

11. Place the HiBind™ DNA Midi column into a clean 15 ml centrifuge tube. **To elute DNA add 0.5-1 ml of Elution Buffer (or 10 mM Tris buffer, pH 8.0) preheated to 60°C-70°C directly onto the HiBind™ DNA Midi column matrix.** Allow to soak for 2-5 min at room temperature. Centrifuge at 6,000 x g for 5 min to collect DNA.

12. Repeat elution step with a second aliquot of Elution Buffer. Typically a total of 200 µg DNA with absorbance ratio (A_{260}/A_{280}) of 1.7-1.9 can be obtained from 0.5 g animal tissue. Yields vary depending on source and quantity of starting material used.

Determination of DNA Quality and Quantity

Dilute a portion of the eluted material approximately 10-20 fold in 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.

Problem	Possible Cause	Suggestions
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.
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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