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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 grams of tissue samples. The special designed buffer systems ensure the optimal lysis of tissue rich in fat, polysaccharides and fibers such as brain, adipose, muscle. This Kit can also 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 is added to provide appropriate binding conditions. The sample is then loaded into the HiBind® DNA Maxi 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 Maxi Kit, except the OB Protease and RNase A can be stored at 22°C-25°C and are guaranteed for at least 24 months from the dated of purchase. OB Protease must be stored at 15-25°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 Maxi column can bind approximately 2.5mg genomic DNA. Using greater than 2g tissue or 5 x10^8 cells is not recommended.

Kit Contents

Product	D5196-00	D5196-01	D5196-02
Purification times	2	10	25
HiBind® DNA Maxi Columns	2	10	25
50 ml Collection tubes	2	10	25
Buffer MTL1	20 ml	100 ml	250 ml
Buffer BL	20 ml	100 ml	250 ml
Buffer HB	22 ml	100 ml	250 ml
OB Protease	**400 µl**	1.5 ml	4 ml
RNase A	30 µl	300 µl	1.2 ml
DNA Wash Buffer	20 ml	2 x 40 ml	3 x 50 ml
Elution Buffer	1 ml	40 ml	50 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 50 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 Maxi Kit protocol.
- Dilute DNA Wash Buffer Concentrate with absolute ethanol as follows and store at room temperature.

D5196-00	Add 80 ml absolute (96%-100%) ethanol.
D5196-01	Add 160 ml (96%-100%) ethanol to each bottle.
D5196-02	Add 200 ml (96%-100%) ethanol to each bottle.

HP Tissue DNA Maxi 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 50ml 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 500 mg tissue. In any event, use no more than 2 grams of tissue per HiBind™ DNA Maxi 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 130 µl OB Protease**. Vortex briefly to mix and incubate at 60°C for a minimum of 2 hours or until the 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 9 ml chloroform:isoamyl alcohol (24:1) and vortex to mix**

at maxi speed for 15 seconds. Centrifuge 4,000 x g for 10 min at room temperature. Carefully transfer the upper aqueous phase to a clean 50 ml microfuge tube. Avoid the milky interface containing contaminants and inhibitors. In most case, around 6 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 upper aqueous solution presented, add 2 ml Buffer MTL1 and vortex to mix. Centrifuge as above and transfer the upper aqueous solution into a new 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 30µl (assuming a sample size of 500 mg) RNase A (25 mg/ml) and incubate at room temperature for 15-60 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 seconds. 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 6ml in step 3, add 6 ml Buffer BL and add 6 ml absolute ethanol.

7. **Apply the mixture from step 6, including any precipitation that may have formed, to an HiBind® DNA Maxi-column assembled in a 50 ml collection tube (supplied).** Centrifuge 4,000 x g for 5 min at room temperature. Discard flow-through liquid. Repeat to apply the remaining mixture to column and centrifuge as above. Discard flow-through liquid.

8. **Place column back into the 50 ml collection tube. Add 9 ml Buffer HB** and centrifuge 4,000 x g for 5 min as above. Discard flow-through liquid and reuse the collecting tube in the next step.

9. **Place column into 50 ml collection tube and wash by adding 12 ml DNA Wash Buffer diluted with absolute ethanol**. Centrifuge 4,000 x g for 5 min as above. Discard flow-through liquid and reuse collection 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 10 ml DNA Wash Buffer diluted with absolute ethanol. Discard liquid and using the empty collection tube**, centrifuge the

column at 4,000 x g for 15 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).

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

12. Repeat elution step with a second aliquot of Elution Buffer. Typically a total of 400 µg DNA with absorbance ratio (A_{260}/A_{280}) of 1.7-1.9 can be obtained from 0.5 gram 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 / OB Protease. 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 / OB Protease. 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 / OB Protease. 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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