



# UltraClean-htp™ 96 Well Tissue DNA Isolation Kit

FOR GENOMIC DNA  
Catalog # 12996-4  
(384 preps)

## Introduction

Use this kit for isolating DNA from 5-25mg of animal tissue samples including difficult tissues like Mouse tails and heart in a 96 Well format.

## Precautions

Please wear gloves at all times when using this product. Avoid all skin contact with reagents in this kit. In case of contact, wash thoroughly with water. Do not ingest. See Material Safety Data Sheets for emergency procedures in case of accidental ingestion or contact. All MSDS information is available upon request (760-929-9911) or on our web site at [www.mobio.com](http://www.mobio.com). Reagents labeled flammable should be kept away from open flames and fire. **WARNING: Solution T5 contains ethanol and it is flammable. This kit is for research purposes only. Not for diagnostic use.**

## Equipment required:

Centrifuge capable of spinning two 96 Well blocks stacked (13 cm x 8 cm x 5.5 cm) at 2500 x g, Multi-channel Pipettor (volumes required 10 µl - 1000 µl)  
Mechanical Shaker that shakes 96 Well Blocks and Adapter (Mo Bio Catalog number: 11996 and 11999)  
Vortex with 3 inch platform  
65-70°C Water Bath  
-20°C Freezer

## Kit Contents (384 Preps)

<u>Description</u>	<u>Amt.</u>
96 Well Bead Plates with Square Well Mat	4 x 96 Well Plates with Beads and Square Well Mat
Tissue Bead Solution	233 ml
RNase A (20 mg/ml)	4.5 ml
DTT	13 ml
Solution T1	26 ml
Solution T2	85 ml
Solution T3	106 ml
Solution T4	550 ml
Solution T5	8 x 30 ml
Solution T6	43 ml
Spin Plates	4
2 ml Collection Plates	4
1 ml Collection Plates	8
0.5 ml Collection Plates	4
Microplates	4
Centrifuge Tape	28
Sealing Tape	8
Elution Sealing Mat	4

## Kit Storage

Store RNase A at 4°C. Store Kit at room temperature for 1 year.

**Technical information: Toll free 1-800-606-6246, or 1-760-929-9911 email: [technical@mobio.com](mailto:technical@mobio.com)**



## Centrifuge Protocol

**Before you start:** Please see precautions on previous page.

There are several things that will make this protocol more efficient to use.

- ◆ First, be sure to measure the centrifuge and rotor you plan to use and be sure they will accommodate the plates used in this kit. For this Centrifuge Protocol it is best to stack a Spin Plate on top of a 0.5 ml Collection Plate. Place this in the plate holder rotor. **DO NOT** start the centrifuge or possible injury or centrifuge damage may occur. Turn the centrifuge by hand slowly and be sure the stacked plates will clear the rotor and centrifuge.
- ◆ Make sure you have a multi-channel pipettor that can accommodate all the required volumes (10 $\mu$ l-1000 $\mu$ l).
- ◆ This protocol assumes you will be processing 192 samples (2-96 well preps). If you plan to process less than this number, divide your samples between two plates evenly so that you always have a balance. See Hints section.
- ◆ Please read all precautions on 96 well plate shaker instruction manual before using it. Failure to do so may result in personal injury or damage to the shaker.
- ◆ Check DTT Solution. If precipitated, heat at 65°C for approximately 5 minutes with periodic mixing until completely dissolved.

### Instructions:

1. Remove Square Well Mat from Bead Plate.
2. **Add 550 $\mu$ l of Bead Solution** to the wells of the Bead Plate.
3. **Add 10  $\mu$ l of RNase A** to each well of 96 Well Bead Plate.
4. **Add 30 $\mu$ l of DTT** to each well of 96 Well Bead Plate.
5. To the Bead Plates add 5-25 mg of tissue.
6. **(Check Solution T1)**. If precipitated, heat to 60°C until dissolved. Mix before using.
7. **Add 60 $\mu$ l of Solution T1**. Place square well mat on plate and seal completely. Gently vortex on a 3 inch platform at medium speed.
8. Centrifuge 96 Well Bead Plate for 3 minutes at 2500 x g.
9. Remove square well mat and **add 200 $\mu$ l of Solution T2**. Secure square well mat tightly to plate.
10. Heat 96 Well Bead Plate for 30 minutes at 65-70°C.
11. Place Bead Plate with square well mat securely fastened, to the 96 Well Plate Shaker (Catalog # 11996). Note: The final order of all components is: Aluminum plate, Square Well mat, 96 Well Bead Plate and Aluminum plate.
12. Shake at speed 30 for 5 minutes.
13. Centrifuge 10 minutes at 2500 x g.
14. Remove and discard square well mat.
15. Transfer the supernatant (500-850  $\mu$ l) to a clean 1ml Collection Plate. **Note:** Volume varies according to tissue type. Supernatant may still contain some tissue particles.
16. **Add 250 $\mu$ l of Solution T3** and apply Sealing Tape to plate.
17. Vortex for 5 seconds and Incubate at -20°C for 5 minutes.
18. Centrifuge the plate for 10 minutes at 2500 x g. Remove and discard Sealing Tape.
19. Avoiding the pellet, transfer entire volume (750-1000  $\mu$ l) of supernatant to a new 1ml Collection Plate.
20. Apply Sealing Tape to plate.
21. Centrifuge 10 min at 2500 x g. Remove and discard Sealing Tape.
22. Transfer entire volume to a 2 ml Collection Plate avoiding any residual pellet.
23. **Add 650  $\mu$ l of Solution T4** to the 2 ml Collection Plate containing the supernatant.
24. **Add a second volume of 650  $\mu$ l of Solution T4** to the same wells of the 2 ml Collection Plate.
25. Pipet up and down to mix.

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26. Place SpinPlate onto a new 0.5 ml Collection Plate.
  27. Load approximately 650µl onto the Spin Plate.
  28. Apply Centrifuge Tape.
  29. Centrifuge for 5 minutes at 2500 x g.
  30. Discard flow through from the 0.5 ml Collection Plate and replace same 0.5 ml Collection Plate beneath Spin Plate.
  31. Remove and discard Centrifuge Tape.
  32. Repeat steps 26 to 30 until all supernatant has been processed. Four repetitions of this process may be required.
  33. **Add 300µl of Solution T5** to Spin Plate.
  34. Apply Centrifuge Tape to Spin Plate.
  35. Centrifuge for 5 minutes at 2500 x g.
  36. Discard flow through in 0.5 ml Collection Plate.
  37. Replace same 0.5 ml Collection Plate beneath Spin Plate.
  38. Remove and discard Centrifuge Tape.
  39. **Add another 300µl of Solution T5** to the Spin Plate.
  40. Apply Centrifuge Tape to Spin Plate.
  41. Centrifuge for 5 minutes at 2500 x g.
  42. Discard flow through in 0.5 ml Collection Plate.
  43. Replace same 0.5 ml Collection Plate beneath Spin Plate.
  44. Centrifuge for 10 minutes at 2500 x g.
  45. Carefully place Spin Plate onto a Microplate.
  46. Remove Centrifuge Tape and discard.
  47. **Add 100µl of Solution T6** to the center of the white filter membrane of Spin Plate.
  48. Apply a new piece of Centrifuge Tape to Spin Plate.
  49. Centrifuge for 5 minutes.
  50. Remove and discard Centrifuge Tape.
  51. Cover wells of Microplate with Elution Sealing Mat provided.
- DNA in the Microplate is now application ready. No further steps are required.  
We recommend storing DNA frozen (-20°C). Solution T6 contains no EDTA.

**Thank you for choosing the UltraClean-htp™ 96 Well Tissue DNA Isolation Kit.**

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## Hints and Troubleshooting Guide

### Processing less than 192 samples (less than 2 full plates)

This protocol assumes you will be processing 192 samples (2-96 well preps). If you plan to process less than this number, divide your samples between two plates evenly.

#### **Distributing samples between two plates:**

Balance the number of samples so centrifugation steps do not damage your centrifuge. It is best to match the total number of samples per plate as well as the orientation. For example, if you use wells A1-A12 in one plate, use those same wells in the second plate.

#### **Multi-Channel pipettors:**

The use of a multi-channel pipettor is advised for maximum efficiency. Most multi-channel pipettors are made to pipet multiples of 8 or 12 samples at a time. Try to purchase one that can pipet a broad range of volumes. (Volumes required 10  $\mu$ l - 650  $\mu$ l).

#### **Reagent reservoirs:**

Use reagent reservoirs for the most efficient pipetting.

#### **Mark used wells:**

Be sure to mark all used wells to prevent reusing wells and cross contamination.

#### **Using the remaining wells of a previously processed plate:**

Be sure to tap the plate several times on the lab bench to force any beads to the bottom of the deep well plate before re-using a plate.

### Amount of tissue to process and viscosity of samples

Depending on tissue type, usually 5-25 mg works well. Initially try lesser amounts (5-10 mg) of tissue when isolating DNA from tissues or cells known to be high in Genomic DNA content. (E.g. spleen, liver, and kidney.) Also, do not exceed  $5 \times 10^6$  cultured cells). It is important not to exceed the capacity of the filters and to keep samples from becoming too viscous.

### To Process Samples from Tissues Embedded in Paraffin (Note: Reagents not provided in kit):

1. Add 1200 $\mu$ l of Xylene to 25mg of tissue or less to a 2 ml centrifuge tube and Vortex for 30 seconds on the highest speed.
2. Centrifuge at 10,000 x g for 5 minutes.
3. Remove supernatant by pipetting.
4. Add 1200 $\mu$ l of 100% Ethanol to sample and gently vortex.
5. Centrifuge again for 5 minutes.
6. Remove residual ethanol carefully by pipetting.
7. Repeat steps 4-6.
8. Incubate tubes open at 37 °C for 15-20 to remove residual ethanol.
9. Now add tissue sample to bead tube and continue with protocol.

### Consistent Sampling of Mouse Tails

When weighing mouse tail samples, the ends of the tails are usually uniform in tissue content. However, when sampling in the region near the base of the tail, care must be taken to ensure consistency. The base will have an inner bone covered with outer flesh, which can be difficult to cut. A new blade should be used to allow cutting a consistent amount of tissue and bone content throughout the samples near the base, and minimize slipping of the blade.



### **Concentrating the DNA**

Your final volume will be 100 $\mu$ l. If this is too dilute for your purposes, you can concentrate by ethanol precipitating in a microcentrifuge tube. Add 4 $\mu$ l of 5M NaCl or 10 $\mu$ l of 3M Sodium Acetate (pH: 5.2) and mix. Then add 200 $\mu$ l of 100% cold ethanol. Mix. Centrifuge at 10,000 x *g* for 5 minutes. Decant all liquid. Dry residual ethanol in a speed vac, dessicator or ambient air. Resuspend precipitated DNA in desired volume.

Alternatively, you can heat plates and evaporate samples down to the desired volume or concentration.

### **DNA floats out of well when loaded on a gel**

You may have inadvertently transferred some residual Solution T5 into the final sample. Prevent this by being careful not to transfer T5 onto the bottom of the Spin Plate. Ethanol precipitation is the best way to remove residues of solution T5. (See concentrating DNA above)

### **Storing DNA**

DNA eluted in Solution T6 (10mM Tris) must be stored at -20°C or it may degrade. DNA can be eluted in TE but the EDTA may inhibit reactions such as PCR and automated sequencing.



**Other UltraClean™ Kits available from MO BIO Laboratories, Inc.**

<u>Kit description</u>	<u>Cat. number</u>
<b>Plasmid Prep Kits</b>	
6 minute Mini Plasmid Prep Kit (100 preps)	12300-100
6 minute Mini Plasmid Prep Kit (250 preps)	12300-250
25-50 ml Plasmid Prep Kit (20 preps)	12700-20
25-50 ml Plasmid Prep Kit (50 preps)	12700-50
250-500 ml Plasmid Prep Kit (10 preps)	12600-10
250-500 ml Plasmid Prep Kit (20 preps)	12600-20
<b>Endotoxin-Free Plasmid Prep Kits</b>	
Endotoxin-free Mini Prep Kit (100 preps)	12311-100
Endotoxin-free Mini Prep Kit (250 preps)	12311-250
Endotoxin-free Midi Prep Kit (10 preps)	12711-10
Endotoxin-free Maxi Prep Kit (10 preps)	12611-10
<b>DNA Purification Kits</b>	
Agarose Gel DNA Purification Kit (300 preps)	12100-300
Agarose Gel-Spin DNA Purification (100 preps)	12400-100
Agarose Gel-Spin DNA Purification (250 preps)	12400-250
PCR Clean-Up Kit (100 preps)	12500-100
PCR Clean-Up Kit (250 preps)	12500-250
<b>DNA Isolation Kits</b>	
DNA Blood Isolation Kit (100 preps)	12000-100
DNA BloodSpin Kit (50 preps)	12200-50
DNA BloodSpin Kit (250 preps)	12200-250
Mega BloodSpin Kit (10 preps)	12210-10
Soil DNA Isolation Kit (50 preps)	12800-50
Soil DNA Isolation Kit (100 preps)	12800-100
Soil DNA Mega Prep Kit (10 preps)	12900-10
Fecal DNA Isolation Kit (50 preps)	12811-50
Fecal DNA Isolation Kit (100 preps)	12811-100
Microbial DNA Isolation Kit (50 preps)	12224-50
Microbial DNA Isolation Kit (250 preps)	12224-250
Plant DNA Isolation Kit (50 preps)	13000-50
Plant DNA Isolation Kit (250 preps)	13000-250
Tissue DNA Isolation Kit (50 preps)	12334-50
Tissue DNA Isolation Kit (250 preps)	12334-250
Water DNA Isolation Kit (10 preps)	14800-10
Water DNA Isolation Kit (25 preps)	14800-25
Forensic DNA Kit- Single prep format (10 preps)	14000-10
Forensic DNA Kit- Single prep format (20 preps)	14000-20
<b>RNA Isolation Kits</b>	
Tissue RNA Isolation Kit (50 preps)	15000-50
Tissue RNA Isolation Kit (250 preps)	15000-250
Plant RNA Isolation Kit (20 preps)	13300-20
Plant RNA Isolation Kit (50 preps)	13300-50
Microbial RNA Isolation Kit (50 preps)	15800-50
Microbial RNA Isolation Kit (250 preps)	15800-250
<b>Growth Media</b>	
TB DRY (1 kg) Terrific Broth powder	12105-1
LB (1 kg) LB powder (Miller)	12106-1
LB Agar (1 kg) LB Agar Powder (Miller)	12107-1

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## Contact Information -

### Technical Information:

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