



# UltraClean™ Tissue DNA Isolation Kit

FOR GENOMIC DNA

| Catalog No. | Quantity  |
|-------------|-----------|
| 12334-50    | 50 Preps  |
| 12334-250   | 250 Preps |

## Instruction Manual

### Introduction

The UltraClean™ Tissue DNA Isolation Kit is ideal for isolating genomic DNA from animal tissues or cultured cells, including rodent tails. Without the use of organic solvents like phenol and chloroform, this kit is safe and user-friendly. The UltraClean™ Tissue DNA Isolation Kit is designed for isolating DNA from 1-25 mg tissue samples.

Fresh or frozen tissue samples are homogenized using bead beating technology to lyse the cells. Lysates are loaded onto a silica spin filter. During a brief spin, the DNA selectively binds to the silica membrane while contaminants pass through. Remaining contaminants and enzyme inhibitors are removed by a wash step. Pure DNA is then eluted into certified, DNA-free Tris buffer.

**This kit is for research purposes only. Not for diagnostic use.**

Version: 02222008

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



### Required Equipment

Microcentrifuge (10,000 x g)  
Pipettor (volumes required 50 – 650 µl)  
Vortex (MO BIO Laboratories catalog# 13111-V)

### Kit Contents

| Components                                    | Kit Catalog #12334-50 |       | Kit Catalog #12334-250 |           |
|---|-----------------------|-------|------------------------|-----------|
|   | Catalog #             | Amt.  | Catalog #              | Amt.      |
| Bead Solution Tubes (contain 550 µl solution) | 12334-50-BST          | 50    | 12334-250-BST          | 250       |
| Solution TD1                                  | 12334-50-1            | 55 ml | 12334-250-1            | 275 ml    |
| Solution TD2                                  | 12334-50-2            | 30 ml | 12334-250-2            | 4 x 30 ml |
| Solution TD3                                  | 12334-50-3            | 6 ml  | 12334-250-3            | 14 ml     |
| Spin Filters                                  | 12334-50-SF           | 50    | 12334-250-SF           | 250       |
| 2 ml Collection Tubes                         | 12334-50-T            | 150   | 12334-250-T            | 750       |

### Kit Storage

Kit reagents and components should be stored at room temperature (15-30°C).

### Precautions

Please wear gloves 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 at [www.mobio.com](http://www.mobio.com). Reagents labeled flammable should be kept away from open flames and sparks.

**WARNING: Solution TD2 contains Ethanol. It is flammable.**

**Make sure the Bead Solution Tubes rotate freely in the centrifuge without rubbing.**



## Protocol

Please wear gloves at all times

1. To the Bead Solution Tubes provided, add 1-25 mg of tissue. **Note:** *If isolating DNA from cells, wash and spin cells down, then add 1 ml of lysis Solution TD1 directly to up to  $5 \times 10^6$  cell pellets and mix thoroughly using a pipettor until solid matter is dispersed. Then proceed directly to step 5; there is no need to use the Bead Solution Tubes when isolating DNA from cultured cells.*  
**Note:** *See Tough Tissue Samples under the Additional Information section at the end of the Instruction Manual for mouse tail and other hard to homogenize samples.*
2. Secure Bead Solution Tubes horizontally using a Vortex Adapter (MO BIO Laboratories Catalog# 13000-V1) or adhere to a flatbed pad with tape, then vortex at maximum speed for 10 minutes. **NOTE: Please see the Additional Information section for other methods of homogenization. The Bead Solution Tubes are compatible with Precellys 24, Fast prep machines, and all bead beater instruments. However, the speed and length of homogenization time are critical to prevent tube breakage.**
3. Remove tubes from adapter and **make sure the Bead Solution Tubes rotate freely in the centrifuge without rubbing.** Spin tubes at 10,000 x g for 1 minute at room temperature and transfer the supernatant to a clean 2 ml Collection Tube (provided).  
**Note:** *With 25 mg of tissue and depending on tissue type, expect 400-450  $\mu$ l of supernatant, which may contain some particles. Solution may be viscous, depending on tissue type and DNA content. See the Additional Information section for help with viscous samples.*
4. Add 450  $\mu$ l (v/v) of Solution TD1, vortex at maximum speed for 5 seconds.
5. Transfer the sample to a Spin Filter (provided) and spin at 10,000 x g at room temperature for 30 seconds. Discard flow through and reload Spin Filter until entire sample has been filtered. **Note:** *A total of two loads for each sample processed are required. Load 600-650  $\mu$ l onto Spin Filter and centrifuge at 10,000 x g for 30 seconds. Discard the flow through and add the remaining supernatant to the Spin Filter and centrifuge at 10,000 x g for 30 seconds.*
6. Add 400  $\mu$ l of Solution TD2 and centrifuge for 30 seconds at 10,000 x g.
7. Discard the flow through.
8. Centrifuge again at 10,000 x g for 1 minute at room temperature to remove residual Solution TD2.
9. Carefully place Spin Filter in a new clean 2 ml Collection Tube (provided). Avoid splashing any Solution TD2 onto the Spin Filter.
10. Add 50  $\mu$ l of Solution TD3 to the center of the white filter membrane.
11. Centrifuge at 10,000 x g at room temperature for 30 seconds.
12. Discard Spin Filter. DNA in the tube is now application ready. No further steps are required.

We recommend storing DNA frozen (-20°C). Solution TD3 contains no EDTA.

**Thank you for choosing the UltraClean™ Tissue DNA Isolation Kit.**

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## Additional Information

### Amount of Tissue to Process and Viscosity of Samples

Depending on the tissue type, usually 1-25 mg works well. Error on the low side when isolating DNA from tissues or cells known to be high in genomic DNA yield (i.e. spleen, liver, and kidney; also, do not exceed  $5 \times 10^6$  cultured cells). It is important not to exceed the capacity of the Spin Filters and to keep samples from becoming too viscous. If viscosity is a problem, and the sample will not flow through the Spin Filter membrane, transfer the supernatant to a fresh tube. Add an equal volume of Solution TD1, vortex, and spin through the Spin Filter membrane using multiple loading to isolate the DNA from the sample.

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

1. Add 1200  $\mu$ l of Xylene to 25 mg of tissue or less to a 2 ml centrifuge tube and vortex for 30 seconds at 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 at 10,000 x g 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 minutes to remove residual Ethanol.
9. Now add tissue sample to a Bead Solution Tube and continue with protocol.

### Tough Tissue Samples

To maximize yields or to process tough samples such as mouse tail, heart tissue, ticks, and other insects, digest samples with a 20 mg/ml Proteinase-K solution (MO BIO Laboratories Catalog#1222-2) as per the following protocol:

1. Add 15  $\mu$ l of a 20 mg/ml Proteinase K solution directly to the Bead Tube containing the tissue sample. **Note:** For mouse tail, 5 mg of tissue is sufficient.
2. Proceed with step two of the protocol by securing the Bead Tubes horizontally in a Vortex Adapter (MO BIO Laboratories Catalog#13000-V1) or adhere to a flatbed pad with tape, then vortex at maximum speed for 10 minutes.
3. Incubate the Bead Tubes at 60° C for 30 minutes.
4. Proceed with step three by spinning samples in a centrifuge at 10,000 x g and transferring the supernatant to clean 2 ml Collection Tubes.

### 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.

## Additional Information cont.

### Other Methods of Homogenization

Some tissues are tough and fibrous which makes lysing the cells difficult; often requiring mechanical homogenization, hand grinding, or freezing and grinding in liquid nitrogen. A vast majority of tissues can be processed for DNA isolation with MO BIO's UltraClean™ Tissue DNA Isolation Kit by means of a simple vortex adapter or hand vortexing for a few minutes. If your goal is to do simple PCR, these methods will produce enough DNA from just about any sample type. However, if your goal is to maximize yield, you may want to consider the following. Tissues such as heart, insects, and ticks are tough to homogenize and thus may require the use of mechanical homogenization. The versatile nature of MO BIO's UltraClean™ Tissue DNA Isolation Kit chemistry makes it compatible with most other methods of homogenization. The following methods have been validated with this kit:

- **Homogenization by hand**

This is achieved through simple grinding with a micro pestle in a microcentrifuge tube. Please note that it is strenuous to handle a large number of samples using this method. Another option is to use a pestle and mortar which is much easier. However, this is effective only when a large amount of tissue is processed. A known amount of material (1–25 mg) can then be transferred to the Bead Solution Tubes and processed as per the protocol. Many researchers use liquid nitrogen to quickly freeze a sample prior to homogenizing by hand. Please see Liquid Nitrogen paragraph below.

- **Homogenization with Precellys® 24**

Up to 24 samples can be homogenized in 2 ml screw cap tubes. This method is fast because the homogenization time is usually only 30 seconds for most tissues and 24 samples can be processed at one time. The following table serves as a guide to determine the optimal speed, number of cycles, and homogenization cycle time: (Note: these times and speeds will also serve for the Fast Prep machine).

| Tissue Type          | Speed    | Number of Cycles | Time/Cycle |
|----------------------|----------|------------------|------------|
| Soft tissues         | 6500 rpm | 1                | 30 seconds |
| Tough Muscle & Heart | 6500 rpm | 2                | 20 seconds |
| Ticks                | 6100 rpm | 3                | 20 seconds |
| Insects              | 6100 rpm | 3                | 20 seconds |

**NOTE:** Homogenization should only be attempted within these guidelines. Exceeding these limits will stress the Bead Solution Tubes and may result in either tube breakage or leaking. Please call Technical Service at 1-800-606-6246 if you wish to explore the possibility of increasing the speed and homogenization time with the Precellys®24.

- **Homogenization with Retsch Shaker**

A Tube Adapter (MO BIO Laboratories Catalog# 11999) is needed in order to process the Bead Solution Tubes in the Retsch Shaker. The recommended speed for this machine is 20 for two cycles of 5 minutes each. The samples are placed in the Tube Adapter and homogenized for 5 minutes and then the Tube Adapter is turned around vertically and the samples are homogenized for another 5 minutes. For tough tissues the recommended time is 20 minutes, 10 minutes on each side of the block, for effective homogenization.

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## Additional Information cont.

- **Liquid Nitrogen**

Liquid nitrogen is another powerful method of homogenization; however there are safety issues involved. Be sure you are trained in the safe use of liquid nitrogen before proceeding or injury could result. If using liquid nitrogen, transfer the material into a microcentrifuge tube that is rated for liquid nitrogen use (call Technical Services if you are not sure) and freeze in liquid nitrogen for up to 10 minutes. Then, using a sterile micro pestle, grind the tissues to a paste as fast as possible. Resuspend the paste in the Bead Solution and transfer the mixture to the Bead Solution Tube and proceed with the protocol.

- **Other Methods**

For other methods of mechanical homogenization please call Technical Service at 800-606-6246.

### **Concentrating the DNA**

Your final volume will be 50  $\mu$ l. If this is too dilute for your purposes, add 5  $\mu$ l of 5M NaCl or 5  $\mu$ l of 3M Sodium Acetate (pH: 5.2) and mix. Then add 100  $\mu$ l of 100% cold Ethanol and mix. It is suggested to incubate the tubes for up to 10 minutes at -20°C and then centrifuge at 10,000 x *g* for 10 minutes. Decant all liquid. Dry residual Ethanol in a speed vac, desiccator, or ambient air. Resuspend precipitated DNA in desired volume.

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

You may have inadvertently transferred some residual Solution TD2 into the final sample. Prevent this by being careful in step 10 not to transfer liquid onto the bottom of the spin filter basket. Ethanol precipitation is the best way to remove residues of Solution TD2. (See "Concentrating the DNA" above)

### **Storing DNA**

DNA eluted in Solution TD3 (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 Quality Products Available from MO BIO Laboratories, Inc.

| <u>Product Description</u>                                  | <u>Catalog No.</u> |
|---|--------------------|
| <b>DNA Isolation Kits</b>                                   |                    |
| UltraClean-htp™ 96 Well Tissue DNA Kit (4 x 96 Preps)       | 12996-4            |
| UltraClean-htp™ 96 Well Tissue DNA Kit (12 x 96 Preps)      | 12996-12           |
| UltraClean™ DNA Blood Isolation Kit (100 preps)             | 12000-100          |
| UltraClean™ BloodSpin™ Kit (50 preps)                       | 12200-50           |
| UltraClean™ BloodSpin™ Kit (250 preps)                      | 12200-250          |
| UltraClean™ Mega BloodSpin™ Kit (10 preps)                  | 12210-10           |
| UltraClean™ Forensic DNA Kit- Single prep format (10 preps) | 14000-10           |
| UltraClean™ Forensic DNA Kit- Single prep format (20 preps) | 14000-20           |
| UltraClean™ Fecal DNA Isolation Kit (50 preps)              | 12811-50           |
| UltraClean™ Microbial DNA Isolation Kit (50 preps)          | 12224-50           |
| <b>RNA Isolation Kits</b>                                   |                    |
| UltraClean™ Tissue RNA Isolation Kit (50 preps)             | 15000-50           |
| UltraClean™ Tissue RNA Isolation Kit (250 preps)            | 15000-250          |
| UltraClean™ Plant RNA Isolation Kit (50 preps)              | 13300-50           |
| UltraClean™ Microbial RNA Isolation Kit (50 preps)          | 15800-50           |
| <b>DNA Purification Kits</b>                                |                    |
| PowerClean™ DNA Clean-Up Kit                                | 12877-50           |
| UltraClean™ 15 DNA Purification Kit (300 preps)             | 12100-300          |
| UltraClean™ GelSpin™ DNA Purification Kit (100 preps)       | 12400-100          |
| UltraClean™ PCR Clean-Up™ Kit (100 preps)                   | 12500-100          |
| <b>Homogenizers</b>   |                    |
| Precellys 24 Homogenizer (120V)                             | 13112              |
| Vortex (120V)   | 13111-V            |
| Vortex Adapter for Genie (2ml tubes x 12)                   | 13000-V1           |

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### Contact Information

#### Technical information:

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For the distributor nearest you, visit our web site at [www.mobio.com/distributors](http://www.mobio.com/distributors)

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