GeneDia

Tissue DNA Extraction Kit MANUAL

GENEDIA™ **life Science Co**.

Product # EK1450R

 # EK14100R

 # EK14200R

 # EK14500R

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Introduction

GENEDIA™ Tissue DNA Extraction Kit provides a rapid and sensitive method for the isolation and purification of total DNA from small input amounts of tissue samples including animals and plant tissues .The purified DNA is of the highest integrity, and can be used in a number of downstream applications.

Kit Specifications

The **GENEDIA™ Tissue DNA Extraction Kit** uses a simple, reliable, phenol-free protocol to isolate DNA from tissue samples. The process involves first lysing the tissue of interest with TiDL, followed by treatment with the provided Proteinase K. The DNA isolation protocol begins with suspending the sample in Absolute Ethanol, containing a strong protein denaturant (the chaotropic salt, guanidine salt), which prevents ribonuclease (DNase) degradation of the DNA. The sample is filtered using a micro-spin containing a silica-based fibre matrix. The nucleic acids in the sample bind to the fibre matrix and then the DNA component is efficiently removed from the matrix-bound sample. The immobilized DNA is washed to remove contaminants, and total DNA is recovered in a final volume of 50 µl.

Kit Components

Components	Product # EK1450R (50 preps)	Product # EK14100R (100 preps)	Product # EK14200R (200 preps)	Product # EK14500R (500 preps)
TiDL (Lysis)	10 ml	20 ml	40 ml	100ml
TiDW1 (Wash 1)	12 ml	22 ml	43 ml	60ml*2
TiDW2 (Wash 2)	7 ml	13 ml	25 ml	35ml*2
TiDE (Elution)	2 ml	4 ml	8 ml	20 ml
Proteinase K	1 ml	2 ml	2 ml*2	10 ml
Spin Columns	50	100	200	250*2
Collection Tubes	50	100	200	250*2
Elution tubes	50	100	200	250*2
Product Insert	1	1	1	1

Storage Conditions

All components of the **GENEDIA™ Tissue DNA Extraction Kit** should be stored at room temperature (20-25 °C) and are stable for 1 year. To prolong the lifetime of Proteinase K, storage at 2–8°C is recommended.

Recommended Equipment and Reagents

- 56-65 °C incubator
- Benchtop microcentrifuge
- Sampler in 100 to 1000 Microliter size

- Sampler tips
- 2 ml microcentrifuge tubes
- Vortex

Precautions and Disclaimers

- Prior to using the protocol, Genetic ID recommends that care be taken in homogenizing the sample in a manner that minimizes the risk of inadvertently contaminating the sample. Contamination can occur using improperly cleaned equipment or using poor laboratory practices during homogenization, weighing and labeling of the subsample.
- The Buffer TiDL contains guanidinium salts, and should be handled with care. Guanidinium salts form highly reactive compounds when combined with bleach, thus care must be taken to properly dispose of any of these solutions.
- All centrifugation steps are carried out in a benchtop microcentrifuge. Various speeds are required for different steps, so please check your microcentrifuge specifications to ensure that it is capable of the proper speeds. All centrifugation steps are performed at room temperature. The correct rpm can be calculated using the formula:

$$RPM = \sqrt{\frac{RCF}{(1.118 \times 10^{-5})\,(r)}}$$

Where RCF = required gravitational acceleration (relative centrifugal force in units of g); r = radius of the rotor in cm; and RPM = the number of revolutions per minute required to achieve the necessary g-force.

- All centrifugation steps are carried out in a benchtop microcentrifuge at 14,000 x g (~ 14,000 RPM) except where noted. All centrifugation steps are performed at room temperature.
- A variable speed centrifuge should be used for maximum kit performance. If a variable speed centrifuge is not available a fixed speed centrifuge can be used, however reduced yields may be observed.

Notes Prior to Use

• Prepare a working concentration of the **TiDW1** by adding:

- 5 ml of 96 - 100% ethanol (not provided) to each of the bottles containing 12 ml of concentrated **TiDW1**. This will give a final volume of 17 ml for **Product # EK1450R**
- 9 ml of 96 - 100% ethanol (not provided) to the supplied bottle containing 22 ml concentrated **TiDW1**. This will give a final volume of 31 ml for **Product # EK14100R**
- 18 ml of 96 - 100% ethanol (not provided) to the supplied bottle containing 43 ml concentrated **TiDW1**. This will give a final volume of 61 ml for **Product # EK14200R**
- 18 ml of 96 - 100% ethanol (not provided) to the supplied bottle containing 43 ml concentrated **TiDW1**. This will give a final volume of 61 ml for **Product # EK14200R**
- 23 ml of 96 - 100% ethanol (not provided) to the supplied bottle containing 54 ml concentrated **TiDW1**. This will give a final volume of 77 ml for **Product # EK14500R**

The labels on the bottles have a box that may be checked to indicate that the ethanol has been added.

• Prepare a working concentration of the **TiDW2** by adding:

- 10 ml of 96 - 100% ethanol (not provided) to each of the bottles containing 7 ml of concentrated **TiDW2**. This will give a final volume of 17 ml for **Product # EK1450R**
- 19 ml of 96 - 100% ethanol (not provided) to the supplied bottle containing 13 ml concentrated **TiDW2**. This will give a final volume of 32 ml for **Product # EK14100R**
- 37 ml of 96 - 100% ethanol (not provided) to the supplied bottle containing 25 ml concentrated **TiDW2**. This will give a final volume of 62 ml for **Product # EK14200R**
- 46 ml of 96 - 100% ethanol (not provided) to the supplied bottle containing 31 ml concentrated **TiDW2**. This will give a final volume of 77 ml for **Product # EK14500R**

The labels on the bottles have a box that may be checked to indicate that the ethanol has been added.

A. Lysate Preparation

1) Determine the amount of tissue by weighing (**5-20mg**), we recommend starting with an input of no more than **20 mg.**
2) Add **200 µl TiDL** and **20 µl Proteinase K**. Grind the tissue thoroughly using a pestle and Mix by vortex.
3) Incubate for **2-3 hours** at **58 °C** (Depending *on the* type *of tissue*), Vortex the tubes occasionally during incubation.
4) After the lysing process wait for the sample to reach room temperature.

   Note: *Most tissue samples will be digested or clarified within the time indicated. If significant amount of visible debris remains, centrifuge the samples at 4,000 x g (~ 3,000 RPM) for 2 minutes and transfer the supernatant to a new microcentrifuge tube (not provided).*

5) After the lysing process wait for the sample to reach room temperature.
6) Add **400 µl** Absolute Ethanol (not provided) and mix by vortexing (2 x 5 s).

B. Binding DNA to Column

7) Assemble a Spin column with collection tube.
8) Transfer onto the column and Incubate for 2 minutes at room temperature.
9) Centrifuge at 8,000 x g (~ 6,000 RPM) for 2 minutes.
10) Discard the flow through. Reassemble the spin column with its collection tube.

    Typically, samples will pass through the columns within ≤ 1 minutes (in less than 1 minute). If the entire volume has not passed, spin for an additional minute.

C. Column Wash

1st wash

11) Add **300 µl Buffer TiDW1** to the GENEDIA DNA Spin column.
12) Centrifuge at **11,000 x g** (~ 10,000 RPM) for **1 minute.**
13) Discard the flow through. Reassemble the spin column with its collection tube.

2nd wash

14) Add **300 µl Buffer TiDW2** to the column and centrifuge for **1 minute** at **11,000 x g** (~ 10,000 RPM).
15) Discard the flow through and reassemble the spin column with its collection tube.
16) Spin the column for 2 minutes in order to thoroughly dry the resin. Discard the collection tube.

D. Elute DNA

17) Place the column into a fresh Elution tube provided with the kit.

18) Add **40 µl** of **TiDE** (preheated at 60°C) to the column. Incubate the assembly at room temperature for **2 minutes**.
19) Centrifuge for **1 minute 12,000 x g** (~12,000 RPM). Note the volume eluted from the column. If the entire volume has not been eluted, spin the column at 14,000 x g (~14,000 RPM) for 1 additional minute.

***Note**: For an improved yield, elute the sample twice and use after concentration process*

E. Storage of DNA

The purified DNA may be stored at –20°C for a few weeks. It is recommended that samples be placed at –70°C for long term storage.

Troubleshooting Guide

Problem	Possible Cause	Solution and Explanation
Poor DNA Recovery	**Incomplete lysis of cells or tissue**	Ensure that the appropriate amount of Digestion Buffer TiDL with Proteinase K added was used. Increase the incubation time
	Column has become clogged	Do not exceed the recommended amounts of starting materials. The amount of starting material may need to be decreased if the column shows clogging below the recommended levels. See also "Clogged Column" below.
	Low DNA content in cells or tissues used	Different tissues and cells have different DNA contents, and thus the expected yield of DNA will vary greatly from these different sources. Please check literature to determine the expected DNA content of your starting material.
Clogged Column	**Insufficient solubilisation of cells or tissues**	Ensure that the appropriate amount of lysis buffer was Used for the amount of cells or tissue.
	Maximum number of cells or amount of tissue exceeds kit specifications	Refer to specifications to determine if amount of starting material falls within kit specifications
	Clarified lysate was not used for the binding step	Ensure that after the lysis step the sample is centrifuged if a significant amount of debris is present, and that only the clarified lysate is used in subsequent steps.
	Centrifuge temperature too low	Ensure that the centrifuge remains at room temperature throughout the procedure. Temperatures below 15°C may cause precipitates to form that can cause the columns to clog.

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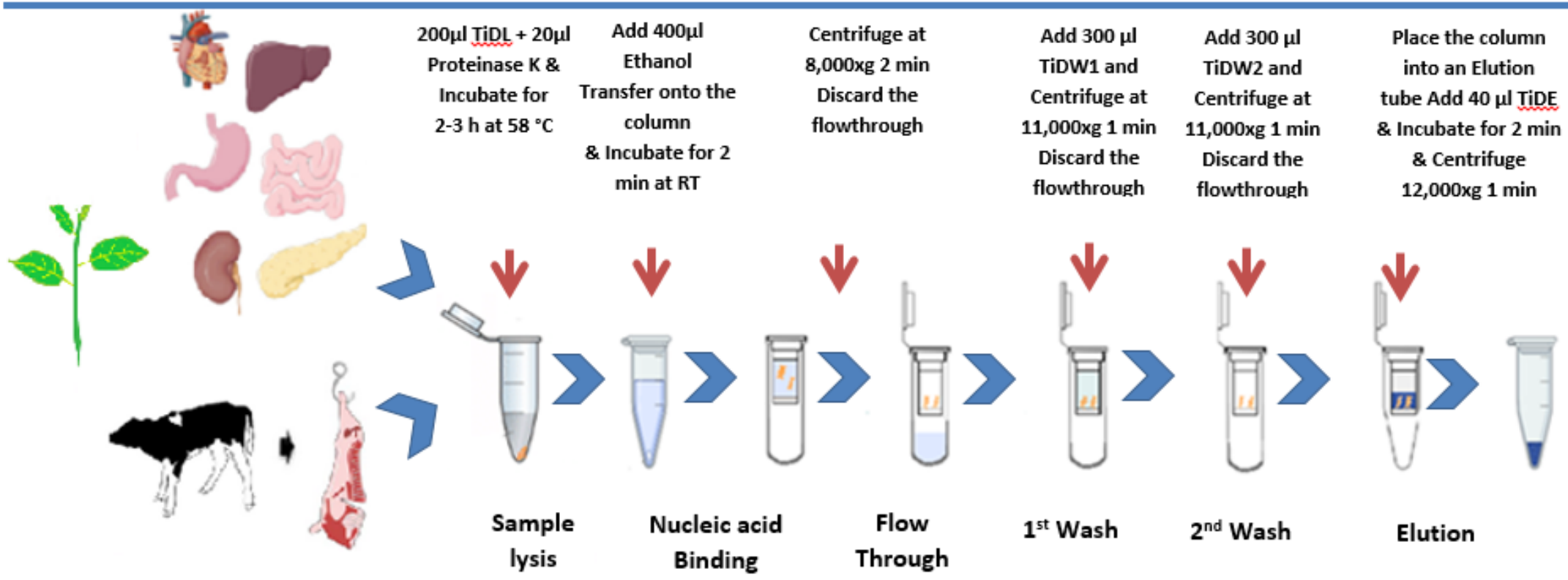
Isolation Method of DNA from tissue samples

Workflow



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