HiYield Genomic DNA Mini Kit (Tissue)

Cat. No.:	YGT50	YGT100	YGT300
Product Name:	HiYield Genomic DNA Mini Kit (Tissue)		
Reactions:	50	100	300
Sample:	Up to 30 mg of Tissue, Up to 25 mg of Paraffin-Embedded Tissue, Buccal Swab and Up to 15 ml of Amniotic Fluid		
Yield:	Up to 50 ug		
Format:	Spin Column		
Operation:	Centrifuge		
Operation Time:	Within 60 Minutes		

Introduction

HiYield Genomic DNA Mini Kit (Tissue) is designed specifically for purifying total DNA (including genomic, mitochondrial and viral DNA) from a variety of animal tissue, paraffin-embedded tissue, buccal swab and amniotic fluid. Provided micropestles can efficiently homogenize tissue samples to shorten the time in the Lysis Step. The entire procedure can be completed within 1 hour without phenol/chloroform extraction or alcohol precipitation. The expected yield of genomic DNA is up to 50 µg and the purified DNA (with approximately 20-30 Kb) is suitable for direct use in PCR or other enzymatic reactions.

Applications

Purified DNA is ready for direct use in PCR, Southern Blotting, Real-Time PCR, AFLP, RFLP, PADP.

Components

ITEM	YGT50	YGT100	YGT300
GT Buffer	30ml	60ml	155ml
GBT Buffer	40ml	60ml	155ml
W1 Buffer	45ml	45ml	130ml
Wash Buffer*	25ml	25ml	50ml
Elution Buffer	30ml	30ml	75ml
Proteinase K**	11mg	22mg	65mg
GT Column	50pcs	100pcs	300pcs
2 ml Collection Tube	100 pcs	200 pcs	600pcs
Micropestle	50pcs	100pcs	300pcs

* Add absolute ethanol to the Wash Buffer prior to initial use (see the bottle label for volume).

**Add dd$H_2$0 to prepare the Proteinase K (vortex to dissolve and spin down) and store at 4ºC (see the bottle label for volume).

Features

Rapid isolation of ready-to-use DNA.
No phenol, chloroform or alcohol.
Simple procedure.

Quality Control

The quality of HiYield Genomic DNA Mini Kit (Tissue) is tested on a lot-to-lot basis by isolation of genomic DNA from 20 mg of mouse liver. Purified DNA is quantified with a spectrophotometer and the yield of genomic DNA is more than 10 ug with A260/A280 ratio 1.7 - 1.9. The purified DNA is checked by electrophoresis.

Caution

GBT Buffer contains guanidine hydrochloride which is a harmful irritant. During operation, always wear a lab coat, disposable gloves, and protective goggles.

References

(1) Vogelstein, B., and Gillespie, D. (1979) Proc. Natl. Acad. Sci. USA 76, 615.

Tissue Protocol

Things to do before starting

1. Add absolute ethanol to the Wash Buffer prior to initial use (see the bottle label for volume).
2. Add ddH_20 to prepare the Proteinase K (vortex to dissolve and spin down) and store at 4ºC (see the bottle label for volume).

Additional requirements: microcentrifuge tubes, absolute ethanol, RNase A (10 mg/ml), ddH_20.

Step 1 Tissue Dissociation	★Cut up to 30 mg of animal tissue (or 0.5 cm of mouse tail) and transfer it to a 1.5 ml microcentrifuge tube. If the tissue has a higher number of cells (eg. spleen or liver), reduce the starting material to 10 mg. ★Use the provided **Micropestle** to grind the tissue to a pulp. ★Add **200 µl of GT Buffer** to the tube and continue to homogenize the sample tissue by grinding.
Step 2 Lysis	★Add **20 µl of Proteinase K** to the sample mixture and mix by vortex. ★Incubate at 60ºC for 30 minutes to lyse the sample. During incubation, invert the tube every 5 minutes. ★Add **200 µl of GBT Buffer** and mix by vortex for 5 seconds. ★Incubate at 70ºC for 20 minutes or until the sample lysate is clear. During incubation, invert the tube every 5 minutes. At this time, preheat the required **Elution Buffer** (200 µl per sample) to 70ºC (for Step 5 DNA Elution). ★If there is insoluble material present following incubation, centrifuge for 2 minutes at full speed and transfer the supernatant to a new 1.5 ml microcentrifuge tube.
	Optional Step: RNA Degradation If RNA free genomic DNA is required, perform this optional step. ★Following 70ºC incubation, add 4 µl of RNase A (10 mg/ml) to the sample lysate and mix by vortex. ★Incubate at room temperature for 5 minutes

Step 3 **DNA** **Binding**	★Add 200 µl of absolute ethanol to the sample lysate and vortex immediately for 10 seconds. If precipitate appears, break it up by pipetting. ★Place a **GT Column** in a **2 ml Collection Tube**. ★Transfer all of the mixture (including any precipitate) to the **GT Column**. ★Centrifuge at full speed for 2 minutes. ★Discard the **2 ml Collection Tube** containing the flow-through and transfer the **GT Column** to a new **2 ml Collection Tube**.
Step 4 **Wash**	★Add **400 µl of W1 Buffer** to the **GT Column**. ★Centrifuge at full speed for 30 seconds. ★Discard the flow-through and place the **GT Column** back in the **2 ml Collection Tube**. ★Add **600 µl of Wash Buffer** (ethanol added) to the **GT Column**. ★Centrifuge at full speed for 30 seconds. ★Discard the flow-through and place the **GT Column** back in the **2 ml Collection Tube**. ★Centrifuge again for 3 minutes at full speed to dry the column matrix.
Step 5 **DNA Elution**	Standard elution volume is 100 µl. If less sample is to be used, reduce the elution volume (30-50 µl) to increase DNA concentration. If higher DNA yield is required, repeat the DNA Elution step to increase DNA recovery and the total elution volume to approximately 200 µl. ★Transfer the dried **GT Column** to a clean 1.5 ml microcentrifuge tube. ★Add **100 µl of preheated Elution Buffer** or TE to the center of the column matrix. ★Let stand for 5 minutes or until the **Elution Buffer** or TE is absorbed by the matrix. ★Centrifuge at full speed for 30 seconds to elute the purified DNA.

Paraffin-Embedded Tissue Protocol

Things to do before starting

1. Add absolute ethanol to the Wash Buffer prior to initial use (see the bottle label for volume).
2. Add ddH_20 to prepare the Proteinase K (vortex to dissolve and spin down) and store at 4ºC (see the bottle label for volume).

Additional requirements: xylene, microcentrifuge tubes, absolute ethanol, RNase A (10 mg/ml), ddH_20.

Step 1 Tissue Dissociation	★Slice small sections (up to 25 mg) from blocks of paraffin-embedded tissue and transfer to a 1.5 ml microcentrifuge tube. ★Add 1 ml of xylene to the tube. Vortex vigorously and incubate at room temperature for approximately 10 minutes. Vortex occasionally during incubation. ★Centrifuge at full speed for 3 minutes. Remove the supernatant. ★Add 1 ml of absolute ethanol to wash the sample pellet and mix by inverting. ★Centrifuge at full speed for 3 minutes. Remove the supernatant. ★Add 1 ml of absolute ethanol to wash the sample pellet again and mix by inverting. ★Centrifuge at full speed for 3 minutes. Remove the supernatant. ★Open the tube and Incubate at 37ºC for 15 minutes to evaporate any ethanol residue.
Step 2 Lysis	★Add **20 µl of Proteinase K** to the sample mixture and mix by vortex. ★Incubate at 60ºC for 30 minutes to lyse the sample. During incubation, invert the tube every 5 minutes. ★Add **200 µl of GBT Buffer** and mix by vortex for 5 seconds. ★Incubate at 70ºC for 20 minutes or until the sample lysate is clear. During incubation, invert the tube every 5 minutes. At this time, preheat the required **Elution Buffer** (200 µl per sample) to 70ºC (for Step 5 DNA Elution). ★If there is insoluble material present following incubation, centrifuge for 2 minutes at full speed and transfer the supernatant to a new 1.5 ml microcentrifuge tube.

	Optional Step: RNA Degradation If RNA free genomic DNA is required, perform this optional step. ★Following 70ºC incubation, add 4 µl of RNase A (10 mg/ml) to the sample lysate and mix by vortex. ★Incubate at room temperature for 5 minutes
Step 3 **DNA** **Binding**	★Add 200 µl of absolute ethanol to the sample lysate and vortex immediately for 10 seconds. If precipitate appears, break it up by pipetting. ★Place a **GT Column** in a **2 ml Collection Tube**. ★Transfer all of the mixture (including any precipitate) to the **GT Column**. ★Centrifuge at full speed for 2 minutes. ★Discard the **2 ml Collection Tube** containing the flow-through and transfer the **GT Column** to a new **2 ml Collection Tube**.
Step 4 **Wash**	★Add **400 µl of W1 Buffer** to the **GT Column**. ★Centrifuge at full speed for 30 seconds. ★Discard the flow-through and place the **GT Column** back in the **2 ml Collection Tube**. ★Add **600 µl of Wash Buffer** (ethanol added) to the **GT Column**. ★Centrifuge at full speed for 30 seconds. ★Discard the flow-through and place the **GT Column** back in the **2 ml Collection Tube**. ★Centrifuge again for 3 minutes at full speed to dry the column matrix.
Step 5 **DNA Elution**	Standard elution volume is 100 µl. If less sample is to be used, reduce the elution volume (30-50 µl) to increase DNA concentration. If higher DNA yield is required, repeat the DNA Elution step to increase DNA recovery and the total elution volume to approximately 200 µl. ★Transfer the dried **GT Column** to a clean 1.5 ml microcentrifuge tube. ★Add **100 µl of preheated Elution Buffer** or TE to the center of the column matrix. ★Let stand for 5 minutes or until the **Elution Buffer** or TE is absorbed by the matrix. ★Centrifuge at full speed for 30 seconds to elute the purified DNA.

Buccal Swab Protocol

Things to do before starting

1. Add absolute ethanol to the Wash Buffer prior to initial use (see the bottle label for volume).
2. Add ddH_20 to prepare the Proteinase K (vortex to dissolve and spin down) and store at 4ºC (see the bottle label for volume).

Additional requirements: Swab (cotton, DACRON or C.E.P. swabs), PBS (phosphate-buffered saline), microcentrifuge tubes, absolute ethanol, ddH_20.

Step 1 Sample Preparation	★Scrape the swab firmly against the inside of each cheek 6-7 times and air-dry the swab. (The person providing the sample should not eat or drink for at least 30 minutes prior to sample collection to avoid contamination.)
Step 2 Lysis	★Add **500 µl of GT Buffer** and **20 µl of Proteinase K** to a 1.5 ml microcentrifuge tube. ★Place the buccal swab into the tube and incubate at 60ºC for 10 minutes. ★Discard the swab and add **500 µl of GBT Buffer** to the lysate. ★Vortex immediately and incubate at 60ºC for 10 minutes. ★At this time, preheat the required **Elution Buffer** (200 µl per sample) in a 60ºC water bath (for Step 5 DNA Elution).
Step 3 DNA Binding	★Add 500 µl of absolute ethanol to the sample lysate and vortex immediately. ★Place a **GT Column** in a **2 ml Collection Tube**. ★Transfer 700 µl of the mixture to the **GT Column**. ★Centrifuge at full speed for 1 minute. ★Repeat the DNA Binding Step by transferring the remaining mixture to the **GT Column**.

Step 4 **Wash**	★Add **400 µl of W1 Buffer** to the **GT Column**. ★Centrifuge at full speed for 30 seconds. ★Discard the flow-through and place the **GT Column** back in the **2 ml Collection Tube**. ★Add **600 µl of Wash Buffer** (ethanol added) to the **GT Column**. ★Centrifuge at full speed for 30 seconds. ★Discard the flow-through and place the **GT Column** back in the **2 ml Collection Tube**. ★Centrifuge again for 3 minutes at full speed to dry the column matrix.
Step 5 **DNA Elution**	Standard elution volume is 100 µl. If less sample is to be used, reduce the elution volume (30-50 µl) to increase DNA concentration. If higher DNA yield is required, repeat the DNA Elution step to increase DNA recovery and the total elution volume to approximately 200 µl. ★Transfer the dried **GT Column** to a clean 1.5 ml microcentrifuge tube. ★Add **100 µl of preheated Elution Buffer** or TE to the center of the column matrix. ★Let stand for 5 minutes or until the **Elution Buffer** or TE is absorbed by the matrix. ★Centrifuge at full speed for 30 seconds to elute the purified DNA.

Amniotic Fluid Protocol

Things to do before starting

1. Add absolute ethanol to the Wash Buffer prior to initial use (see the bottle label for volume).
2. Add ddH_20 to prepare the Proteinase K (vortex to dissolve and spin down) and store at 4ºC (see the bottle label for volume).

Additional requirements: centrifuge tubes, microcentrifuge tubes, absolute ethanol, RNase A (10 mg/ml), ddH_20.

Step 1 Cell Harvesting/ Prelysis	★Transfer approximately 10 ml (up to 15 ml) of amniotic fluid to a 15 ml centrifuge tube. ★Centrifuge for 3 minutes at full speed and discard the supernatant.
Step 2 Lysis	★Add **200 µl of GT Buffer** to resuspend the pellet and transfer the mixture to a 1.5 ml microcentrifuge tube. ★Add **10 µl of Proteinase K (10 mg/ml)** to the sample mixture and mix by vortex. ★Incubate at 60ºC for 30 minutes to lyse the sample. During incubation, invert the tube every 5 minutes. ★Add **200 µl of GBT Buffer** to the 1.5 ml microcentrifuge tube and mix by vortex for 5 seconds. ★Incubate at 70ºC for 20 minutes or until the sample lysate is clear. During incubation, invert the tube every 5 minutes. ★At this time, preheat the required **Elution Buffer** (200 µl per sample) to 70ºC. (for Step 5 DNA Elution)
	Optional Step: RNA Degradation If RNA free genomic DNA is required, perform this optional step. ★Following 70ºC incubation, add 5 µl of RNase A (10 mg/ml) to the sample lysate and mix by vortex. ★Incubate at room temperature for 5 minutes

Step 3 **DNA** **Binding**	★Add 200 µl of absolute ethanol to the sample lysate and vortex immediately for 10 seconds. If precipitate appears, break it up by pipetting. ★Place a **GT Column** in a **2 ml Collection Tube**. ★Transfer all of the mixture (including any precipitate) to the **GT Column**. ★Centrifuge at full speed for 1 minute. ★Discard the **2 ml Collection Tube** containing the flow-through and transfer the **GT Column** to a new **2 ml Collection Tube**.
Step 4 **Wash**	★Add **400 µl of W1 Buffer** to the **GT Column**. ★Centrifuge at full speed for 30 seconds. ★Discard the flow-through and place the **GT Column** back in the **2 ml Collection Tube**. ★Add **600 µl of Wash Buffer** (ethanol added) to the **GT Column**. ★Centrifuge at full speed for 30 seconds. ★Discard the flow-through and place the **GT Column** back in the **2 ml Collection Tube**. ★Centrifuge again for 3 minutes at full speed to dry the column matrix.
Step 5 **DNA Elution**	Standard elution volume is 100 µl. If less sample is to be used, reduce the elution volume (30-50 µl) to increase DNA concentration. If higher DNA yield is required, repeat the DNA Elution step to increase DNA recovery and the total elution volume to approximately 200 µl. ★Transfer the dried **GT Column** to a clean 1.5 ml microcentrifuge tube. ★Add **100 µl of preheated Elution Buffer** or TE to the center of the column matrix. ★Incubate the **GT Column** at 37ºC in an incubator for 10 minutes. ★Centrifuge at full speed for 1 minute to elute the purified DNA.

Hairs That Include Follical Cells Protocol

Things to do before starting

1. Add absolute ethanol to the Wash Buffer prior to initial use (see the bottle label for volume).
2. Add dd$H_2$0 to prepare the Proteinase K (vortex to dissolve and spin down) and store at 4ºC (see the bottle label for volume).

Additional requirements: microcentrifuge tubes, absolute ethanol, RNase A (10 mg/ml), dd$H_2$0.

Step 1 Sample Lysis	★Add 10 hairs that include follical cells to a 2 ml Collection Tube. ★Add **200 µl of GT Buffer and 20 ul of Proteinase K** to the 2 ml Collection Tube and mix by shaking. (be sure the buffer completely cover the sample) ★Incubate at 60ºC for 30 minutes to lyse the sample. During incubation, shaking the 2 ml Collection Tube every 5 minutes. ★Add **200 ul of GBT Buffer** and mix vigorously . ★Incubate at 70ºC for 20 minutes and during incubation, shaking the 2 ml Collection Tube every 5 minutes. ★At this time, preheat the required **Elution Buffer** (100 µl per sample) to 70ºC (for DNA Elution step). ★After incubation, centrifuge for 5 minutes at 14,000 x g and transfer the supernatant to a new 2 ml Collection Tube.
	Optional Step: RNA Degradation If RNA free genomic DNA is required, perform this optional step. ★Following 70ºC incubation, add 4 µl of RNase A (10 mg/ml) to the sample lysate and mix by vortex. ★Incubate at room temperature for 5 minutes
Step 2 DNA Binding	★Add 200 µl of absolute ethanol to the sample lysate in the 2 ml Collection Tube from the previous step. ★Mix immediately by pipetting 5-10 times. ★Place a **GT Column** on a new 2 ml Collection Tube. ★Transfer the lysate mixture to the **GT Column**. ★Centrifuge for 5 minutes at 14,000 x g.

Step 3 **Wash**	★Add **400 µl of W1 Buffer** to the **GT Column**. ★Centrifuge for 5 minutes at 14,000 x g. ★Add **600 µl of Wash Buffer** (ethanol added) to the **GT Column** to wash again. ★Centrifuge for 5 minutes at 14,000 x g. ★Discard the flow-through and place the **GT Column** back on the 2 ml Collection Tube. ★Centrifuge for 10 minutes at 14,000 x g to remove any ethanol residue
Step 4 **DNA Elution**	Standard elution volume is 100 µl. If less sample is to be used, reduce the elution volume (50-100 µl) to increase DNA concentration. If higher DNA yield is required, repeat the DNA Elution step to increase DNA recovery and the total elution volume to approximately 200 µl. ★Transfer the **GT Column** to a clean **1.5 ml microcentrifuge tube** ★Add **50 µl of preheated Elution Buffer** or TE to the center of the **GT Column**. ★Let stand for 3 minutes or until the **Elution Buffer** or TE is absorbed by the matrix. ★Centrifuge for 5 minutes at 14,000 x g to elute the purified DNA. ★Add **50 µl of preheated Elution Buffer** or TE to the center of the **GT Column**. ★Let stand for 3 minutes or until the **Elution Buffer** or TE is absorbed by the matrix. ★Centrifuge for 5 minutes at 14,000 x g to elute the purified DNA. ★Use 5-10 µl for PCR or qPCR (dependent on how many volume of Elution buffer you use)

Troubleshooting

Problem	Possible Reason/ Solution
Column clogged	**Too much sample was used.** ★Reduce sample volume or separate into multiple tubes.
	Sample tissue was not lysed completely. ★Add additional Proteinase K and extend the incubation time in the Lysis Step. ★Following the Lysis Step, centrifuge for 2 minutes at full speed to remove sample debris. Transfer the supernatant to a new microcentrifuge tube and proceed with the DNA Binding Step.
	Precipitate was formed at DNA Binding Step. ★Reduce the sample material. ★Prior to loading the column, break up precipitate in ethanol-added lysate.
Low yield	**Sample tissue was not lysed completely** ★Add additional Proteinase K and extend the incubation time in the Lysis Step.
	Column was clogged at DNA Binding Step ★Following the Lysis Step, remove the insoluble debris by centrifugation. ★Prior to loading the column, break up the precipitate in the ethanol added lysate.
	Incorrect DNA Elution Step. ★Ensure that Elution Buffer was added and absorbed to the center of GT Column matrix completely.
	Incomplete DNA Elution. ★Elute twice to increase yield.
Eluted DNA does not perform well in downstream applications.	**Residual ethanol contamination.** ★Following the wash step, dry GT Column with additional centrifugation at full speed for 5 minutes or incubation at 60℃ for 5 minutes.
	RNA contamination. ★Perform Optional RNA degradation Step.
	Genomic DNA was degraded. ★Use fresh samples or freeze fresh samples in liquid nitrogen immediately and store at -80ºC.