Geneaid

Instruction Manual
Ver. 02.21.17
For Research Use Only

Geneaid™ DNA Isolation Kit

GEB100, GEB01K, GEB01K+
GEC150, GEC1.5K, GEC1.5K+
GET150, GET1.5K, GET1.5K+
GEE150, GEE1.5K, GEE1.5K+

Advantages

Sample: tissue, rodent tails, ear punches, fresh or frozen blood, cultured cells, bacteria
Yield: high yield, high quality DNA (A260/A280 = 1.8-2.0)
Format: scalable DNA precipitation method
Kit Storage: dry at room temperature (15-25°C), Proteinase K and RNase A should be stored at 4°C for extended periods, Lysozyme should be stored at -20°C for extended periods

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Geneaid Biotech Ltd.
Tel: 886 2 26960999 · Fax: 886 2 26960599 · www.geneaid.com · info@geneaid.com

Introduction

The Geneaid™ DNA Isolation Kit offers a simple and gentle reagent DNA precipitation method for isolating high molecular weight genomic, mitochondrial or viral DNA suitable for archiving or sensitive downstream applications. This highly versatile solution based system can be scaled proportionately in order to satisfy larger sample volumes providing a convenient sample-storage procedure with minimal hands on time. Initially cells are lysed in the presence of detergents and a proprietary DNA stabilization solution followed by RNase A treatment. Once proteins and other contaminants are removed DNA is precipitated then rehydrated. The high quality extracted DNA is ready for use in a variety of downstream applications.

Quality Control

Geneaid™ DNA Isolation Kits are tested on a lot-to-lot basis according to Geneaid's ISO-certified quality management system. Genomic DNA is isolated from 300 µl of fresh whole human blood. The isolated DNA (5-15 µg with an A260/A280 ratio of 1.8–2.0) is quantified with a spectrophotometer and analyzed by electrophoresis.

Components and Storage

RBC Lysis Buffer, Cell Lysis Buffer, Protein Removal Buffer, Gram+ Buffer, DNA Hydration Buffer should be stored dry at room temperature (15-25ºC) for up to 2 years. Proteinase K and RNase A should be stored at 4ºC for extended periods. Add ddH_2O to Proteinase K (see the bottle label for volume) then vortex to ensure Proteinase K is completely disolved. Check the box on the bottle. Once it is disolved completely, centrifuge for a few seconds to spin the mixture down. The Proteinase K mixture should be stored at 4ºC for extended periods. Lysozyme should be stored at -20ºC for extended periods.

Table 1. 100 rxns (GEB100) or 1,000 rxns (GEB01K) can be performed when processing 1 ml of whole blood.

Geneaid™ Blood Kit	GEB003	GEB100	GEB01K	GEB01K+
Volume of blood processed per kit	3 ml	100 ml	1000 ml	1000 ml
RBC Lysis Buffer	12 ml	360 ml	500 ml x 7	500 ml x 7
Cell Lysis Buffer	3 ml	100 ml	500 ml x 2	500 ml x 2
Protein Removal Buffer	1 ml	40 ml	400 ml	400 ml
DNA Hydration Buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0)	1 ml	50 ml	500 ml	500 ml
RNase A (10 mg/ml)	25 µl	550 µl	Not included	5 ml

Table 2. 150 rxns (GEC150) or 1,500 rxns (GEC1.5K) can be performed when processing 5 x 10^6 cells.

Geneaid™ Cultured Cell Kit	GEC005	GEC150	GEC1.5K	GEC1.5K+
Number of cells processed per kit	**2 x 10^7**	**6 x 10^8**	**6 x 10^9**	**6 x 10^9**
Cell Lysis Buffer	3 ml	100 ml	1000 ml	1000 ml
Protein Removal Buffer	1 ml	40 ml	400 ml	400 ml
DNA Hydration Buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0)	1 ml	50 ml	500 ml	500 ml
RNase A (10 mg/ml)	25 µl	550 µl	Not included	5 ml

Table 3. 150 rxns (GET150) or 1,500 rxns (GET1.5K) can be performed when processing 20 mg of tissue.

Geneaid™ Tissue Kit	GET005	GET150	GET1.5K	GET1.5K+
Amt. of tissue processed per kit	**100 mg**	**3.3 g**	**33 g**	**33 g**
Cell Lysis Buffer	3 ml	100 ml	1000 ml	1000 ml
Protein Removal Buffer	1 ml	40 ml	400 ml	400 ml
DNA Hydration Buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0)	1 ml	50 ml	500 ml	500 ml
RNase A (10 mg/ml)	25 µl	550 µl	Not included	5 ml
Proteinase K (add ddH_2O)	1 mg (0.1 ml)	11 mg x 2 (1.1 ml)	65 mg x 3 (6.5 ml) 11 mg x 1 (1.1 ml)	65 mg x 3 (6.5 ml) 11 mg x 1 (1.1 ml)

Table 4. 150 rxns (GEE150) or 1,500 rxns (GEE1.5K) can be performed when processing 1 x 10^9 bacteria cells.

Geneaid™ Bacteria Kit	GEE005	GEE150	GEE1.5K	GEE1.5K+
Number of cells processed per kit	**5 x 10^9**	**1.5 x 10^{11}**	**1.5 x 10^{12}**	**1.5 x 10^{12}**
Cell Lysis Buffer	3 ml	100 ml	1000 ml	1000 ml
Protein Removal Buffer	1 ml	40 ml	400 ml	400 ml
DNA Hydration Buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0)	1 ml	50 ml	500 ml	500 ml
Gram+ Buffer	1.5 ml	30 ml	150 ml	150 ml
RNase A (10 mg/ml)	25 µl	550 µl	Not included	5 ml
Lysozyme	8 mg	130 mg	Not included	610 mg x 2

Scaling Large Sample Volumes

Table 5. Whole blood reagent volumes

Blood volume	300 µl	500 µl	1 ml	3 ml	10 ml
Number of white cells	2.1 x 10^6	3.5 x 10^6	7 x 10^6	2.1 x 10^7	7 x 10^7
Tube size	1.5 ml	15 ml	15 ml	50 ml	50 ml
RBC Lysis Buffer	900 µl	1.5 ml	3 ml	9 ml	30 ml
Cell Lysis Buffer	300 µl	500 µl	1 ml	3 ml	10 ml
RNase A (10 mg/ml)	1.5 µl	2.5 µl	5 µl	15 µl	50 µl
Protein Removal Buffer	100 µl	167 µl	333 µl	1 ml	3.33 ml
Isopropanol	300 µl	500 µl	1 ml	3 ml	10 ml
70% ethanol	300 µl	500 µl	1 ml	3 ml	10 ml
DNA Hydration Buffer	100 µl	100 µl	100 µl	300 µl	1 ml

Table 6. Compromised whole blood reagent volumes

Blood volume	3 ml	10 ml
Number of white cells	2.1 x 10^7	7 x 10^7
Tube size	15 ml	50 ml
RBC Lysis Buffer	9 ml	30 ml
Cell Lysis Buffer	3 ml	10 ml
RNase A (10 mg/ml)	15 µl	50 µl
Protein Removal Buffer	1.35 ml	4.5 ml
Isopropanol	4 ml	13.5 ml
70% ethanol	3 ml	10 ml
DNA Hydration Buffer	200 µl	500 µl

Table 7. Cultured cell reagent volumes

Cell number	0.5-1 x 10^6	3-5 x 10^6	3-5 x 10^7
Tube size	1.5 ml	1.5 ml	15 ml
Cell Lysis Buffer	150 µl	600 µl	6 ml
RNase A (10 mg/ml)	1 µl	3 µl	30 µl
Protein Removal Buffer	50 µl	200 µl	2 ml
Isopropanol	150 µl	600 µl	6 ml
70% ethanol	150 µl	600 µl	6 ml
DNA Hydration Buffer	50 µl	100 µl	200 µl

Table 8. Tissue reagent volumes

Tissue weight	1-5 mg	10-20 mg	150-200 mg
Tube size	1.5 ml	1.5 ml	15 ml
Cell Lysis Buffer	100 µl	600 µl	6 ml
Proteinase K (ddH_2O added)	3 µl	12 µl	120 µl
RNase A (10 mg/ml)	1 µl	3 µl	30 µl
Protein Removal Buffer	33 µl	200 µl	2 ml
Isopropanol	100 µl	600 µl	6 ml
70% ethanol	100 µl	600 µl	6 ml
DNA Hydration Buffer	100 µl	100 µl	200 µl

Table 9. Gram (-) negative/Gram (+) positive bacteria reagent volumes

Gram (-) Bacterial cell №	0.5-1.5 x 10^9	Gram (+) Bacteria cell №	0.5-1.5 x 10^9
Tube size	1.5 ml	Tube size	1.5 ml
Cell Lysis Buffer	300 µl	Gram+ Buffer/Lysozyme	100 µl/0.8 mg
RNase A (10 mg/ml)	3.5 µl	Cell Lysis Buffer	300 µl
Protein Removal Buffer	100 µl	RNase A (10 mg/ml)	3.5 µl
Isopropanol	300 µl	Protein Removal Buffer	100 µl
70% ethanol	300 µl	Isopropanol	300 µl
DNA Hydration Buffer	100 µl	70% ethanol	300 µl
		DNA Hydration Buffer	100 µl

300 µl Whole Blood Protocol Procedure

Please read the entire instruction manual prior to starting the Protocol Procedure.

1. RBC Lysis

Transfer 900 µl of RBC Lysis Buffer and 300 µl of whole blood into a 1.5 ml microcentrifuge tube then mix by inverting. Do not vortex. Incubate for 5 minutes at room temperature then centrifuge at 3,000 x g for 5 minutes to form a leukocyte (white blood cell) pellet. Carefully remove the supernatant, retaining approximately 50 µl of residual buffer and leukocyte pellet. Vortex the tube until the leukocyte pellet is completely resuspended in the residual buffer.

2. Lysis

Add 300 µl of Cell Lysis Buffer to the tube then mix by vortex. Incubate at 60ºC for at least 10 minutes to ensure the sample lysate is clear and homogenous. During incubation, invert the tube every 3 minutes.

Optional RNA Removal Step

Following 60ºC incubation, add 1.5 µl of RNase A (10 mg/ml) to the sample lysate then mix by vortex. Incubate at room temperature for 5 minutes.

3. Protein Removal

Add 100 µl of Protein Removal Buffer to the sample lysate then vortex immediately for 10 seconds. Centrifuge at 14-16,000 x g for 3 minutes to form a tight, dark brown, protein pellet. NOTE: If the pellet is loose, incubate on ice for 5 minutes followed by centrifugation at 14-16,000 x g for another 3 minutes.

4. DNA Precipitation

Transfer the supernatant to a clean 1.5 ml microcentrifuge tube then add 300 µl of isopropanol and mix well by gently inverting 20 times. Centrifuge at 14-16,000 x g for 5 minutes then carefully discard the supernatant and add 300 µl of 70% ethanol to wash the pellet. Centrifuge at 14-16,000 x g for 3 minutes then carefully discard the supernatant and air-dry the pellet for 10 minutes.

5. DNA Rehydration

Add 100 µl of DNA Hydration Buffer then gently vortex for 10 seconds. Incubate at 60ºC for 5-10 minutes to dissolve the DNA pellet. During incubation, tap the bottom of the tube to promote DNA rehydration.

3 ml Whole Blood Protocol Procedure

Please read the entire instruction manual prior to starting the Protocol Procedure.

1. RBC Lysis

Transfer 9 ml of RBC Lysis Buffer and 3 ml of whole blood into a 15 ml centrifuge tube then mix by inverting. Do not vortex. Incubate for 5 minutes at room temperature then centrifuge at 3,000 x g for 5 minutes to form a leukocyte (white blood cell) pellet. Carefully remove the supernatant, retaining approximately 300 µl of residual buffer and leukocyte pellet. Vortex the tube until the leukocyte pellet is completely resuspended in the residual buffer.

2. Lysis

Add 3 ml of Cell Lysis Buffer to the tube then mix by vortex. Incubate at 60ºC for at least 10 minutes to ensure the sample lysate is clear and homogenous. During incubation, invert the tube every 3 minutes.

Optional RNA Removal Step

Following 60ºC incubation, add 15 µl of RNase A (10 mg/ml) to the sample lysate then mix by vortex. Incubate at room temperature for 10 minutes.

3. Protein Removal

Add 1 ml of Protein Removal Buffer to the sample lysate then vortex immediately for 10 seconds. Centrifuge at 2,000-3,000 x g for 5 minutes to form a tight, dark brown, protein pellet.

NOTE: If the pellet is loose, incubate on ice for 5 minutes followed by centrifugation at 3,000-6,000 x g for another 5 minutes.

4. DNA Precipitation

Transfer the supernatant to a clean 15 ml centrifuge tube then add 3 ml of isopropanol and mix well by gently inverting 20 times. Centrifuge at 2,000-3,000 x g for 5 minutes and carefully discard the supernatant then add 3 ml of 70% ethanol to wash the pellet. Centrifuge at 2,000-3,000 x g for 3 minutes then carefully discard the supernatant and air-dry the pellet for 10 minutes.

5. DNA Rehydration

Add 300 µl of DNA Hydration Buffer then gently vortex for 10 seconds. Incubate at 60ºC for 30-60 minutes to dissolve the DNA pellet. During incubation, tap the bottom of the tube to promote DNA rehydration.

10 ml Whole Blood Protocol Procedure

Please read the entire instruction manual prior to starting the Protocol Procedure.

1. RBC Lysis

Transfer 30 ml of RBC Lysis Buffer and 10 ml of whole blood into a 50 ml centrifuge tube then mix by inverting. Do not vortex. Incubate for 5 minutes at room temperature then centrifuge at 3,000 x g for 5 minutes to form a leukocyte (white blood cell) pellet. Carefully remove the supernatant, retaining approximately 300 µl of residual buffer and leukocyte pellet. Vortex the tube until the leukocyte pellet is completely resuspended in the residual buffer.

2. Lysis

Add 10 ml of Cell Lysis Buffer to the tube then mix by vortex. Incubate at 60ºC for at least 10 minutes to ensure the sample lysate is clear and homogenous. During incubation, invert the tube every 3 minutes.

Optional RNA Removal Step

Following 60ºC incubation, add 50 µl of RNase A (10 mg/ml) to the sample lysate then mix by vortex. Incubate at room temperature for 10 minutes.

3. Protein Removal

Add 3.33 ml of Protein Removal Buffer to the sample lysate then vortex immediately for 10 seconds. Centrifuge at 2,000-3,000 x g for 5 minutes to form a tight, dark brown, protein pellet.

NOTE: If the pellet is loose, incubate on ice for 5 minutes followed by centrifugation at 3,000-6,000 x g for another 5 minutes.

4. DNA Precipitation

Transfer the supernatant to a clean 50 ml centrifuge tube then add 10 ml of isopropanol and mix well by gently inverting 20 times. Centrifuge at 2,000-3,000 x g for 5 minutes then carefully discard the supernatant and add 10 ml of 70% ethanol to wash the pellet. Centrifuge at 2,000-3,000 x g for 3 minutes then carefully discard the supernatant and air-dry the pellet for 10 minutes.

5. DNA Rehydration

Add 1 ml of DNA Hydration Buffer then gently vortex for 10 seconds. Incubate at 60ºC for 30-60 minutes to dissolve the DNA pellet. During incubation, tap the bottom of the tube to promote DNA rehydration.

10 ml Compromised Whole Blood Protocol Procedure

Please read the entire instruction manual prior to starting the Protocol Procedure.

1. RBC Lysis

Transfer 30 ml of RBC Lysis Buffer and 10 ml of whole blood into a 50 ml centrifuge tube then mix by inverting. Do not vortex. Incubate for 5 minutes at room temperature then centrifuge at 3,000 x g for 5 minutes to form a leukocyte (white blood cell) pellet. Carefully remove the supernatant, retaining approximately 300 µl of residual buffer and leukocyte pellet. Vortex the tube until the leukocyte pellet is completely resuspended in the residual buffer.

2. Lysis

Add 10 ml of Cell Lysis Buffer to the tube then mix by vortex. Incubate at 60ºC for at least 10 minutes to ensure the sample lysate is clear and homogenous. During incubation, invert the tube every 3 minutes.

Optional RNA Removal Step

Following 60ºC incubation, add 50 µl of RNase A (10 mg/ml) to the sample lysate then mix by vortex. Incubate at room temperature for 10 minutes.

3. Protein Removal

Add 4.5 ml of Protein Removal Buffer to the sample lysate then vortex immediately for 10 seconds. Centrifuge at 2,000-3,000 x g for 5 minutes to form a tight, dark brown, protein pellet.

NOTE: If the pellet is loose, incubate on ice for 5 minutes followed by centrifugation at 3,000-6,000 x g for another 5 minutes.

4. DNA Precipitation

Transfer the supernatant to a clean 50 ml centrifuge tube then add 13.5 ml of isopropanol and mix well by gently inverting 20 times. Centrifuge at 2,000-3,000 x g for 5 minutes then carefully discard the supernatant and add 10 ml of 70% ethanol to wash the pellet. Centrifuge at 2,000-3,000 x g for 3 minutes then carefully discard the supernatant and air-dry the pellet for 10 minutes.

5. DNA Rehydration

Add 500 µl of DNA Hydration Buffer then gently vortex for 10 seconds. Incubate at 60ºC for 30-60 minutes to dissolve the DNA pellet. During incubation, tap the bottom of the tube to promote DNA rehydration.

3-5 x 10^6 Cultured Cell Protocol Procedure

Please read the entire instruction manual prior to starting the Protocol Procedure.

1. Sample Preparation

Adherent Cultured Animal Cells (trypsinize cells prior to harvesting)

Remove the culture medium and wash cells in PBS. Aspirate PBS and add 0.10-0.25% Trypsin in PBS. Once cells detach add the medium then transfer to a 1.5 ml microcentrifuge tube. Proceed with Suspension Cultured Animal cells.

Suspension Cultured Animal Cells

Transfer cells (3-5 x 10^6) to a 1.5 ml microcentrifuge tube then centrifuge for 5 minutes at 300 x g. Discard the supernatant retaining approximately 50 µl of residual buffer and cell pellet. Vortex the tube until the cell pellet is completely resuspended in the residual buffer.

2. Lysis

Add 600 µl of Cell Lysis Buffer to the tube then mix by vortex. Incubate at 60ºC for at least 10 minutes to ensure the sample lysate is clear/homogenous. During incubation, invert the tube every 3 minutes.

Optional RNA Removal Step

Following 60ºC incubation, add 3 µl of RNase A (10 mg/ml) to the sample then mix by vortex. Incubate at room temperature for 5 minutes.

3. Protein Removal

Add 200 µl of Protein Removal Buffer then vortex immediately for 10 seconds. Centrifuge at 14-16,000 x g for 3 minutes to form a tight pellet.

NOTE: If the pellet is loose, incubate on ice for 5 minutes followed by centrifugation at 14-16,000 x g for another 3 minutes.

4. DNA Precipitation

Transfer the supernatant to a new 1.5 ml microcentrifuge tube then add 600 µl of isopropanol and mix well by gently inverting 20 times. Centrifuge at 14-16,000 x g for 5 minutes then carefully discard the supernatant and add 600 µl of 70% ethanol to wash the pellet. Centrifuge at 14-16,000 x g for 3 minutes then carefully discard the supernatant and air-dry the pellet for 10 minutes.

5. DNA Rehydration

Add 100 µl of DNA Hydration Buffer then gently vortex for 10 seconds. Incubate at 60ºC for 30-60 minutes to dissolve the DNA pellet. During incubation, tap the bottom of the tube to promote DNA rehydration.

3-5 x 10^7 Cultured Cell Protocol Procedure

Please read the entire instruction manual prior to starting the Protocol Procedure.

1. Sample Preparation

Adherent Cultured Animal Cells (trypsinize cells prior to harvesting)

Remove the culture medium and wash cells in PBS. Aspirate PBS and add 0.10-0.25% Trypsin in PBS. Once cells detach add the medium then transfer to a 15 ml centrifuge tube. Proceed with Suspension Cultured Animal cells.

Suspension Cultured Animal Cells

Transfer cells (3-5 x 10^7) to a 15 ml centrifuge tube then centrifuge for 5 minutes at 300 x g. Discard the supernatant retaining approximately 50 µl of residual buffer and cell pellet. Vortex the tube until the cell pellet is completely resuspended in the residual buffer.

2. Lysis

Add 6 ml of Cell Lysis Buffer to the tube then mix by vortex. Incubate at 60ºC for at least 10 minutes to ensure the sample lysate is clear/homogenous. During incubation, invert the tube every 3 minutes.

Optional RNA Removal Step

Following 60ºC incubation, add 30 µl of RNase A (10 mg/ml) to the sample then mix by vortex. Incubate at room temperature for 5 minutes.

3. Protein Removal

Add 2 ml of Protein Removal Buffer then vortex immediately for 10 seconds. Centrifuge at 2,000-3,000 x g for 5 minutes to form a tight pellet.

NOTE: If the pellet is loose, incubate on ice for 5 minutes followed by centrifugation at 3,000-6,000 x g for another 5 minutes.

4. DNA Precipitation

Transfer the supernatant to a new 15 ml centrifuge tube then add 6 ml of isopropanol and mix well by gently inverting 20 times. Centrifuge at 2,000-3,000 x g for 5 minutes then carefully discard the supernatant and add 6 ml of 70% ethanol to wash the pellet. Centrifuge at 2,000-3,000 x g for 3 minutes then carefully discard the supernatant and air-dry the pellet for 10 minutes.

5. DNA Rehydration

Add 200 µl of DNA Hydration Buffer then gently vortex for 10 seconds. Incubate at 60ºC for 30-60 minutes to dissolve the DNA pellet. During incubation, tap the bottom of the tube to promote DNA rehydration.

0.5-1.5 x 10^9 Bacteria Protocol Procedure

Please read the entire instruction manual prior to starting the Protocol Procedure.

Transfer the Gram (-) negative bacteria culture (0.5-1.5 x 10^9) to a 1.5 ml microcentrifuge tube then centrifuge at 14-16,000 x g for 1 minute. Discard the supernatant. Proceed to step 1 Lysis.

Gram (+) Positive Bacteria Sample Preparation

Transfer the bacteria culture (0.5-1.5 x 10^9) to a 1.5 ml microcentrifuge tube. Centrifuge for 1 minute at 14-16,000 x g then discard the supernatant. Transfer the required volume of Gram+ Buffer (100 µl/sample) to a 15 ml centrifuge tube. Add Lysozyme (0.8 mg/100 µl) to Gram+ Buffer (in the 15 ml centrifuge tube) then vortex to completely dissolve the Lysozyme. Transfer 100 µl of Gram+ Buffer (make sure Lysozyme was added) to the bacteria pellet. Resuspend the pellet by shaking vigorously or pipette. Incubate at room temperature for 10-20 minutes. During incubation, invert the tube every 2-3 minutes.

1. Lysis

Add 300 µl of Cell Lysis Buffer to the tube then mix by vortex. Incubate at 60ºC for at least 10 minutes to ensure the sample lysate is clear/homogenous. During incubation, invert the tube every 3 minutes.

Optional RNA Removal Step

Following 60ºC incubation, add 1.5 µl of RNase A (10 mg/ml) to the sample lysate then mix by vortex. Incubate at room temperature for 5 minutes.

2. Protein Removal

Add 100 µl of Protein Removal Buffer to the sample lysate then vortex immediately for 10 seconds. Centrifuge at 14-16,000 x g for 3 minutes to form a tight pellet.

NOTE: If the pellet is loose, incubate on ice for 5 minutes followed by centrifugation at 14-16,000 x g for another 3 minutes.

3. DNA Precipitation

Transfer the supernatant to a new 1.5 ml centrifuge tube then add 300 µl of isopropanol and mix well by gently inverting 20 times. Centrifuge at 14-16,000 x g for 5 minutes then carefully discard the supernatant and add 300 µl of 70% ethanol to wash the pellet. Centrifuge at 14-16,000 x g for 3 minutes then carefully discard the supernatant and air-dry the pellet for 10 minutes.

4. DNA Rehydration

Add 100 µl of DNA Hydration Buffer then gently vortex for 10 seconds. Incubate at 60ºC for 30-60 minutes to dissolve the DNA pellet. During incubation, tap the bottom of the tube to promote DNA rehydration.

10-20 mg Tissue Protocol Procedure

Please read the entire instruction manual prior to starting the Protocol Procedure.

1. Tissue Dissociation

Transfer 10-20 mg of tissue (0.5 cm of mouse tail) to a 1.5 ml microcentrifuge tube and use a micropestle to grind the tissue a few times. Add 600 µl of Cell Lysis Buffer to the tube and continue to homogenize the sample tissue with grinding.

2. Lysis

Add 12 µl of Proteinase K to the tube then mix by vortex. Incubate at 60ºC for 30-60 minutes or until the tissue has dissolved completely. During incubation, invert the tube periodically.

Optional RNA Removal Step

Following 60ºC incubation, add 3 µl of RNase A (10 mg/ml) to the sample lysate then mix by vortex. Incubate at room temperature for 5 minutes.

3. Protein Removal

Add 200 µl of Protein Removal Buffer to the sample lysate then vortex immediately for 10 seconds. Centrifuge at 14-16,000 x g for 3 minutes to form a tight pellet.

NOTE: If the pellet is loose, incubate on ice for 5 minutes followed by centrifugation at 14-16,000 x g for another 3 minutes.

4. DNA Precipitation

Transfer the supernatant to a clean 1.5 ml microcentrifuge tube then add 600 µl of isopropanol and mix well by gently inverting 20 times. Centrifuge at 14-16,000 x g for 5 minutes then carefully discard the supernatant and add 600 µl of 70% ethanol to wash the pellet. Centrifuge at 14-16,000 x g for 3 minutes then carefully discard the supernatant and air-dry the pellet for 10 minutes.

5. DNA Rehydration

Add 100 µl of DNA Hydration Buffer then gently vortex for 10 seconds. Incubate at 60ºC for 30-60 minutes to dissolve the DNA pellet. During incubation, tap the bottom of the tube to promote DNA rehydration.

150-200 mg Tissue Protocol Procedure

Please read the entire instruction manual prior to starting the Protocol Procedure.

1. Tissue Dissociation

Freeze 150-200 mg of tissue with liquid nitrogen then grind to a fine powder using a mortar and pestle. Add 6 ml of Cell Lysis Buffer to the morter and continue to homogenize the sample tissue with grinding. Transfer the homogenized sample to a 15 ml centrifuge tube.

2. Lysis

Add 120 µl of Proteinase K to the tube then mix by vortex. Incubate at 60ºC for 30-60 minutes or until the tissue has dissolved completely. During incubation, invert the tube periodically.

Optional RNA Removal Step

Following 60ºC incubation, add 30 µl of RNase A (10 mg/ml) to the sample lysate then mix by vortex. Incubate at room temperature for 10 minutes.

3. Protein Removal

Add 2 ml of Protein Removal Buffer to the sample lysate then vortex immediately for 10 seconds. Centrifuge at 2,000-3,000 x g for 5 minutes to form a tight pellet.

NOTE: If the pellet is loose, incubate on ice for 5 minutes followed by centrifugation at 3,000-6,000 x g for another 5 minutes.

4. DNA Precipitation

Transfer the supernatant to a clean 15 ml centrifuge tube then add 6 ml of isopropanol and mix well by gently inverting 20 times. Centrifuge at 2,000-3,000 x g for 5 minutes then carefully discard the supernatant and add 6 ml of 70% ethanol to wash the pellet. Centrifuge at 2,000-3,000 x g for 3 minutes then carefully discard the supernatant and air-dry the pellet for 10 minutes.

5. DNA Rehydration

Add 200 µl of DNA Hydration Buffer then gently vortex for 10 seconds. Incubate at 60ºC for 30-60 minutes to dissolve the DNA pellet. During incubation, tap the bottom of the tube to promote DNA rehydration.

Troubleshooting

Improper sample homogenization.

Yield and quality of DNA will be higher when fresh samples or samples which have been flash frozen and stored at -20ºC or -70ºC are used. Fresh blood is recommended. However, frozen or blood treated with anticoagulants (EDTA etc.) can also be used. Increased storage length decreases DNA yield.

Incomplete protein removal.

A solid protein pellet must be formed following centrifugation in Step 3.

RNA contamination.

Perform the optional RNA removal step.

Slow DNA rehydration.

In step 5, tap the bottom of the tube occasionally to facilitate DNA rehydration. If the DNA pellet is too dry, incubate at 60ºC for 60 minutes or at room temperature overnight.

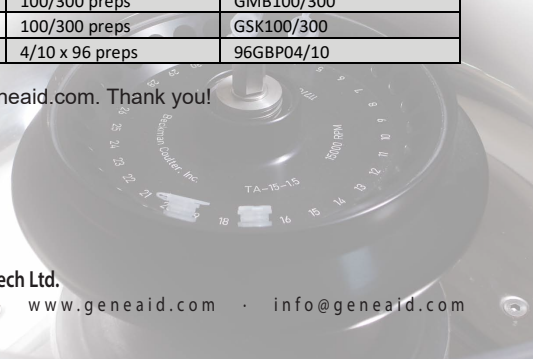
Eluted DNA does not perform well in downstream applications.

Increase DNA pellet drying time to ensure residual ethanol is completely evaporated.

Related DNA Extraction Products

Product	Package Size	Catalogue Number
Genomic DNA Mini Kit (Blood/Cultured Cell)	100/300 preps	GB100/300
Genomic DNA Midi Kit (Blood/Cultured Cell)	25 preps	GDI25
Genomic DNA Maxi Kit (Blood/Cultured Cell)	10/25 preps	GDM10/25
Genomic DNA Mini Kit (Tissue)	50/100/300 preps	GT050/100/300
gSYNC™ DNA Extraction Kit	50/100/300 preps	GS050/100/300
Genomic DNA Mini Kit (Plant)	100 preps	GP100
Geneaid™ DNA Isolation Kit (Blood)	100/1,000 rxns	GEB100/01K(+)
Geneaid™ DNA Isolation Kit (Bacteria)	300/3,000 rxns	GEE300/03K(+)
Geneaid™ DNA Isolation Kit (Tissue)	150/1,500 rxns	GET150/1.5K(+)
Geneaid™ DNA Isolation Kit (Cultured Cell)	150/1,500 rxns	GEC150/1.5K(+)
GENEzol™ DNA Reagent Plant	100/200 rxns	GR100/200
Presto™ Mini gDNA Yeast Kit	100/300 preps	GBY100/300
Presto™ Mini gDNA Bacteria Kit	100/300 preps	GBB100/101/300/301
Geneius™ Micro DNA Extraction Kit	100/300 preps	GMB100/300
Presto™ Buccal Swab gDNA Extraction Kit	100/300 preps	GSK100/300
Presto™ 96 Well Blood Genomic DNA Extraction Kit	4/10 x 96 preps	96GBP04/10

For additional product information please visit www.geneaid.com. Thank you!



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