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HiYield Genomic DNA Micro Kit

Cat. No.:	YGM100	YGM300
Product Name:	HiYield Genomic DNA Micro Kit	
Reactions:	100	300
Sample:	1-100 µl of small volume blood samples, 6 mm diameter of blood card punches (dried blood spots) and 1 ml of urine	
Yield:	300 ng of pure genomic DNA from 6 mm diameter of blood spots	
Elution Volume:	50-100 µl	
Format:	Spin Column	
Operation:	Centrifuge	
Operation Time:	Within 20 Minutes	

Description

HiYield Genomic DNA Micro Kit provides a fast and economical method for purification of genomic and mitochondrial DNA from small volumes of whole blood, dried blood spots and urine. The entire procedure can be completed in 20 minutes without phenol/chloroform extraction or alcohol precipitation, with an average DNA yield of 300 ng from 6 mm diameter of blood spots and with a minimum DNA yield of 300 ng from 10 µl of whole blood. Purified DNA, with approximately 20-30 kb, is suitable for direct use in PCR or other enzymatic reactions.

Features

1. Reliable purification of high-quality genomic DNA from small sample volumes within 20 minutes.
2. Consistent and high yields, with an average DNA yield of 300 ng from 6 mm diameter of blood spots.
3. Complete removal of all contaminants for sensitive downstream applications.
4. No phenol, chloroform or alcohol.

Applications

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

Quality Control

The quality of HiYield Genomic DNA Micro Kit is tested on a lot-to-lot basis by isolation of genomic DNA from 10 µl of human whole blood. The purified DNA is quantified with a spectrophotometer and the yield of genomic DNA is more than 300 ng with A260/A280 ratio 1.8 - 2.0. The purified DNA is checked by electrophoresis.

Contents

Item	YGM100	YGM300
GM1 Powder*	1 mg	1 mg
GM2 Buffer	30 ml	75 ml
GM3 Buffer	30 ml	75 ml
Proteinase K**	22 mg	65 mg
W1 Buffer	45 ml	130 ml
Wash Buffer (concentrated)***	25 ml	50 ml
Elution Buffer	75 ml	150 ml
GD Column	100 pcs	300 pcs
2 ml Collection Tube	200 pcs	600 pcs

* Add 1 ml of Elution Buffer to GM1 Powder then vortex to ensure GM1 Powder is completely dissolved to obtain a working solution of 1 µg/µl. Check the box on the bottle label showing Elution Buffer is added. Once GM1 Powder is dissolved completely, centrifuge for a few seconds to spin down the mixture. Divide the GM1 Buffer into convenient volumes in several RNase-free 1.5 ml microcentrifuge tubes. The GM1 Buffer should be stored at -20ºC. Do not freeze and thaw GM1 Buffer more than 3 times.

**Add ddH_20 (pH7.0-8.5) to Proteinase K (see the bottle label for volume) then vortex to ensure Proteinase K is completely dissolved. Check the box on the bottle showing ddH_20 is added. Once it is dissolved completely, centrifuge for a few seconds to spin down the mixture. For extended periods, store the ddH_20 and Proteinase K mixture at 4ºC. Use only fresh ddH_20 as ambient CO_2 can quickly cause acidification.

*** Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle showing absolute ethanol is added. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.

Storage

HiYield Genomic DNA Micro Kit shall be shipped and stored dry at room temperature (15-25℃). With proper storage, HiYield Genomic DNA Micro Kit can be stored for up to 12 months without showing any deduction in performance and quality. For extended periods, store Proteinase K at 4ºC or -20 ºC.

Amounts of Starting Material and the Protocol to be Followed:

Sample	Amount	Protocol	Page
Whole Blood	1-100 µl	Whole Blood	3
Blood Card Punches (dried blood spots)	6 mm diameter	Dried Blood Spot	5
Urine	1 ml (up to 10 ml)	Urine	7

Whole Blood Protocol

Please read the entire instruction manual prior to starting.

Things to Do before Starting:

1. Add 1 ml of Elution Buffer to GM1 Powder then vortex to ensure GM1 Powder is completely dissolved to obtain a working solution of 1 µg/µl. Check the box on the bottle label showing Elution Buffer is added. Once GM1 Powder is dissolved completely, centrifuge for a few seconds to spin down the mixture. Divide the GM1 Buffer into convenient volumes in several RNase-free 1.5 ml microcentrifuge tubes. The GM1 Buffer should be stored at -20ºC. Do not freeze and thaw GM1 Buffer more than 3 times.
2. Add ddH_20 (pH7.0-8.5) to Proteinase K (see the bottle label for volume) then vortex to ensure Proteinase K is completely dissolved. Check the box on the bottle showing ddH_20 is added. Once it is dissolved completely, centrifuge for a few seconds to spin down the mixture. For extended periods, store the ddH_20 and Proteinase K mixture at 4ºC. Use only fresh ddH_20 as ambient CO_2 can quickly cause acidification.
3. Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle showing absolute ethanol is added. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.

Additional Requirements: RNase-free 1.5 ml microcentrifuge tubes.

Caution: During operation, always wear a lab coat, disposable gloves and protective goggles.

Step 1 Buffer Preparation	1. Make sure GM1 Buffer, Proteinase K mixture and Wash Buffer are prepared according to the above instructions. 2. Transfer 1 µl of GM1 Buffer and 200 µl of GM2 Buffer per sample to a RNase-free 1.5 ml microcentrifuge tube and vortex shortly to mix. The mixture, GM12 Buffer, is for use in the Cell Lysis step.
Step 2 Cell Lysis	1. Transfer 1-100 µl of whole blood to a 1.5 ml microcentrifuge tube and add GM3 Buffer to a final volume of 200 µl. Add 20 µl of Proteinase K (make sure ddH_2O was added) then mix by vortex. Incubate at 60ºC for 5 minutes to lyse the sample. Make sure the sample and GM3 Buffer are mixed thoroughly to yield a homogenous solution. 2. Add 200 µl of GM12 Buffer (make sure 1 µl of GM1 Buffer was added) and mix by vortex. Incubate at 60ºC for 5 minutes. Inverting the sample occasionally during incubation will facilitate Proteinase K digestion and cell lysis. Incubating with a shaking incubator is another convenient option. 3. During incubation, transfer the required volume of Elution Buffer (200 µl/sample) to a 1.5 ml microcentrifuge tube and heat to 60ºC (for Step 5 DNA Elution).

Step 3 DNA Binding	1. Add 200 µl of absolute ethanol to the sample lysate and mix IMMEDIATELY by shaking vigorously for 10 seconds. If precipitate appears, break it up with a pipette. 2. Place a GD Column in a 2 ml Collection Tube. Transfer all of the mixture (including any insoluble precipitate) to the GD Column. Centrifuge at 14,000-16,000 x g for 3 minutes. 3. If the mixture did not flow through the GD Column membrane, increase the centrifuge time until it passes completely. Discard the 2 ml Collection Tube containing the flow-through then transfer the GD Column to a new 2 ml Collection Tube. NOTE: It is important that the lysate and ethanol are mixed thoroughly to yield a homogeneous solution.
Step 4 Wash	1. Add 400 µl of W1 Buffer to the GD Column. Centrifuge at 14,000-16,000 x g for 30 seconds then discard the flow-through. Place the GD Column back in the 2 ml Collection Tube. 2. Add 600 µl of Wash Buffer (absolute ethanol added) to the GD Column. Centrifuge at 14,000-16,000 x g for 30 seconds then discard the flow-through. Place the GD Column back in the 2 ml Collection Tube. 3. Centrifuge again for 3 minutes at 14,000-16,000 x g to dry the column matrix. NOTE: Additional centrifugation at 14,000-16,000 x g for 5 minutes or incubation at 60ºC for 5 minutes will completely dry the GD Column to avoid any residual ethanol carryover and ensure the most effective downstream applications.
Step 5 DNA Elution	1. Transfer the dried GD Column to a clean 1.5 ml microcentrifuge tube. 2. Add 100 µl[1] of pre-heated Elution Buffer[2], TE Buffer[3] or water[4] into the **CENTER** of the column matrix. Let stand for at least 3 minutes to allow Elution Buffer, TE Buffer or water to be completely absorbed. 3. Centrifuge at 14,000-16,000 x g for 1 minute to elute the purified DNA. NOTE: [1] Standard elution volume is 100 µl. To increase DNA concentration, reduce the elution volume to 50-100 µl. To increase DNA recovery, repeat the DNA Elution step. [2] Ensure that Elution Buffer (10 mM Tris-HCl, pH8.5 at 25ºC) is added into the center of the GD Column matrix and is completely absorbed. [3]Using TE (10 mM Tris-HCl, 1 mM EDTA, pH8.0) for elution is beneficial as EDTA preserves DNA for long term storage. However, EDTA will affect PCR and other sensitive downstream applications. [4]If using water for elution, ensure the water pH is between 7.0 and 8.5. ddH_2O should be fresh as ambient CO_2 can quickly cause acidification. DNA Eluted in water should be stored at -20ºC to avoid degradation.

Dried Blood Spot Protocol

Please read the entire instruction manual prior to starting.

Things to Do before Starting:

1. Add 1 ml of Elution Buffer to GM1 Powder then vortex to ensure GM1 Powder is completely dissolved to obtain a working solution of 1 µg/µl. Check the box on the bottle label showing Elution Buffer is added. Once GM1 Powder is dissolved completely, centrifuge for a few seconds to spin down the mixture. Divide the GM1 Buffer into convenient volumes in several RNase-free 1.5 ml microcentrifuge tubes. The GM1 Buffer should be stored at -20ºC. Do not freeze and thaw GM1 Buffer more than 3 times.
2. Add ddH_20 (pH7.0-8.5) to Proteinase K (see the bottle label for volume) then vortex to ensure Proteinase K is completely dissolved. Check the box on the bottle showing ddH_20 is added. Once it is dissolved completely, centrifuge for a few seconds to spin down the mixture. For extended periods, store the ddH_20 and Proteinase K mixture at 4ºC. Use only fresh ddH_20 as ambient CO_2 can quickly cause acidification.
3. Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle showing absolute ethanol is added. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.

Additional Requirements: RNase-free 1.5 ml microcentrifuge tubes.

Caution: During operation, always wear a lab coat, disposable gloves and protective goggles.

Step 1 Buffer Preparation	1. Make sure GM1 Buffer, Proteinase K mixture and Wash Buffer are prepared according to the above instructions. 2. Transfer 1 µl of GM1 Buffer and 200 µl of GM2 Buffer per sample to a RNase-free 1.5 ml microcentrifuge tube and vortex shortly to mix. The mixture, GM12 Buffer, is for use in the Cell Lysis step.
Step 2 Cell Lysis	1. Cut 6 mm diameter punches from a dried blood spot and transfer to a 1.5 ml microcentrifuge tube. Add 200 µl of GM3 Buffer and 20 µl of Proteinase K (make sure ddH_2O was added) then mix by vortex (be sure the sample is completely immersed in the buffer). Incubate at 60ºC for 30 minutes to lyse the sample. During incubation, vortex the tube every 10 minutes. Mixing thoroughly yields a homogenous solution. 2. Add 200 µl of GM12 Buffer (make sure 1 µl of GM1 Buffer was added) and mix by vortex. Incubate at 60ºC for 20 minutes. During incubation, vortex the tube every 10 minutes. Inverting the sample facilitates Proteinase K digestion and cell lysis. 3. During incubation, transfer the required volume of Elution Buffer (200 µl/sample) to a 1.5 ml microcentrifuge tube and heat to 60ºC (for Step 5 DNA Elution). 4. After incubation, briefly centrifuge the tube and transfer the supernatant to a new 1.5 ml microcentrifuge tube.

Step 3 DNA Binding	1. Add 200 µl of absolute ethanol to the sample lysate and then mix thoroughly by vortex for 10 seconds. If precipitate appears, break it up as much as possible with a pipette. 2. Place a GD Column in a 2 ml Collection Tube. Transfer all of the mixture (including any insoluble precipitate) to the GD Column. Centrifuge at 14,000-16,000 x g for 3 minutes. 3. If the mixture did not flow through the GD Column membrane, increase the centrifuge time until it passes completely. Discard the 2 ml Collection Tube containing the flow-through then transfer the GD Column to a new 2 ml Collection Tube. NOTE: It is important that the lysate and ethanol are mixed thoroughly to yield a homogeneous solution.
Step 4 Wash	1. Add 400 µl of W1 Buffer to the GD Column. Centrifuge at 14,000-16,000 x g for 30 seconds then discard the flow-through. Place the GD Column back in the 2 ml Collection Tube. 2. Add 600 µl of Wash Buffer (absolute ethanol added) to the GD Column. Centrifuge at 14,000-16,000 x g for 30 seconds then discard the flow-through. Place the GD Column back in the 2 ml Collection Tube. 3. Centrifuge again for 3 minutes at 14,000-16,000 x g to dry the column matrix. NOTE: Additional centrifugation at 14,000-16,000 x g for 5 minutes or incubation at 60ºC for 5 minutes will completely dry the GD Column to avoid any residual ethanol carryover and ensure the most effective downstream applications.
Step 5 DNA Elution	1. Transfer the dried GD Column to a clean 1.5 ml microcentrifuge tube. 2. Add 100 µl[1] of pre-heated Elution Buffer[2], TE Buffer[3] or water[4] into the **CENTER** of the column matrix. Let stand for at least 3 minutes to allow Elution Buffer, TE Buffer or water to be completely absorbed. 3. Centrifuge at 14,000-16,000 x g for 1 minute to elute the purified DNA. NOTE: [1] Standard elution volume is 100 µl. To increase DNA concentration, reduce the elution volume to 50-100 µl. To increase DNA recovery, repeat the DNA Elution step. [2] Ensure that Elution Buffer (10 mM Tris-HCl, pH8.5 at 25ºC) is added into the center of the GD Column matrix and is completely absorbed. [3]Using TE (10 mM Tris-HCl, 1 mM EDTA, pH8.0) for elution is beneficial as EDTA preserves DNA for long term storage. However, EDTA will affect PCR and other sensitive downstream applications. [4]If using water for elution, ensure the water pH is between 7.0 and 8.5. ddH_2O should be fresh as ambient CO_2 can quickly cause acidification. DNA Eluted in water should be stored at -20ºC to avoid degradation.

Urine Protocol

Please read the entire instruction manual prior to starting.

Things to Do before Starting:

1. Add 1 ml of Elution Buffer to GM1 Powder then vortex to ensure GM1 Powder is completely dissolved to obtain a working solution of 1 µg/µl. Check the box on the bottle label showing Elution Buffer is added. Once GM1 Powder is dissolved completely, centrifuge for a few seconds to spin down the mixture. Divide the GM1 Buffer into convenient volumes in several RNase-free 1.5 ml microcentrifuge tubes. The GM1 Buffer should be stored at -20ºC. Do not freeze and thaw GM1 Buffer more than 3 times.
2. Add ddH_20 (pH7.0-8.5) to Proteinase K (see the bottle label for volume) then vortex to ensure Proteinase K is completely dissolved. Check the box on the bottle showing ddH_20 is added. Once it is dissolved completely, centrifuge for a few seconds to spin down the mixture. For extended periods, store the ddH_20 and Proteinase K mixture at 4ºC. Use only fresh ddH_20 as ambient CO_2 can quickly cause acidification.
3. Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle showing absolute ethanol is added. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.

Additional Requirements:

RNase-free 1.5 ml microcentrifuge tubes.

For male urine samples: Dithiothreitol (DTT), for increasing DNA yield from male urine samples containing sperm cells. If DTT is required, please refer to cat. no. YDT500, Dithiothreitol (DTT) 500µl.

Caution: During operation, always wear a lab coat, disposable gloves and protective goggles.

Step 1 Buffer Preparation	1. Make sure GM1 Buffer, Proteinase K mixture and Wash Buffer are prepared according to the above instructions. 2. Transfer 1 µl of GM1 Buffer and 200 µl of GM2 Buffer per sample to a RNase-free 1.5 ml microcentrifuge tube and vortex shortly to mix. The mixture, GM12 Buffer, is for use in the Cell Lysis step.
Step 2 Sample Preparation	1. Transfer 1 ml of urine to a 1.5 ml microcentrifuge tube then centrifuge at 6,000 x g for 2 minutes. Discard the supernatant then add 500 µl of Elution Buffer to the pellet and vortex for 5 seconds. NOTE: If using 2-10 ml of urine samples, transfer the sample to a 15 ml centrifuge tube and centrifuge at 6,000 x g for 2 minutes. Discard the supernatant; add 500 µl of Elution Buffer then vortex for 5 seconds. Transfer the sample to a 1.5 ml microcentrifuge tube. 2. Centrifuge at 6,000 x g for 2 minutes, discard the supernatant.

Step 3 Cell Lysis	1. Add 200 µl of GM3 Buffer and 20 µl of Proteinase K (make sure ddH_2O was added) then mix by vortex for 10 seconds. To increase DNA yield from male urine samples containing sperm cells, add 20 µl of 1M DTT. If DTT is required, please refer to cat. no. YDT500, Dithiothreitol (DTT). 2. Incubate at 60ºC for 30 minutes to lyse the sample. During incubation, vortex the tube every 10 minutes. Mixing thoroughly yields a homogenous solution. 3. Add 200 µl of GM12 Buffer (make sure 1 µl of GM1 Buffer was added) and mix by vortex. Incubate at 60ºC for 20 minutes. During incubation, vortex the tube every 10 minutes. Inverting the sample facilitates Proteinase K digestion and cell lysis. 4. During incubation, transfer the required volume of Elution Buffer (200 µl/sample) to a 1.5 ml microcentrifuge tube and heat to 60ºC (for Step 6 DNA Elution).
Step 4 DNA Binding	1. Add 200 µl of absolute ethanol to the sample lysate and then mix thoroughly by vortex for 10 seconds. If precipitate appears, break it up as much as possible with a pipette. 2. Place a GD Column in a 2 ml Collection Tube. Transfer all of the mixture (including any insoluble precipitate) to the GD Column. Centrifuge at 14,000-16,000 x g for 3 minutes. 3. If the mixture did not flow through the GD Column membrane, increase the centrifuge time until it passes completely. Discard the 2 ml Collection Tube containing the flow-through then transfer the GD Column to a new 2 ml Collection Tube. NOTE: It is important that the lysate and ethanol are mixed thoroughly to yield a homogeneous solution.
Step 5 Wash	1. Add 400 µl of W1 Buffer to the GD Column. Centrifuge at 14,000-16,000 x g for 30 seconds then discard the flow-through. Place the GD Column back in the 2 ml Collection Tube. 2. Add 600 µl of Wash Buffer (absolute ethanol added) to the GD Column. Centrifuge at 14,000-16,000 x g for 30 seconds then discard the flow-through. Place the GD Column back in the 2 ml Collection Tube. 3. Centrifuge again for 3 minutes at 14,000-16,000 x g to dry the column matrix. NOTE: Additional centrifugation at 14,000-16,000 x g for 5 minutes or incubation at 60ºC for 5 minutes will completely dry the GD Column to avoid any residual ethanol carryover and ensure the most effective downstream applications.

Step 6 DNA Elution	1. Transfer the dried GD Column to a clean 1.5 ml microcentrifuge tube. 2. Add 100 µl[1] of pre-heated Elution Buffer[2], TE Buffer[3] or water[4] into the **CENTER** of the column matrix. Let stand for at least 3 minutes to allow Elution Buffer, TE Buffer or water to be completely absorbed. 3. Centrifuge at 14,000-16,000 x g for 1 minute to elute the purified DNA. NOTE: [1] Standard elution volume is 100 µl. To increase DNA concentration, reduce the elution volume to 50-100 µl. To increase DNA recovery, repeat the DNA Elution step. [2] Ensure that Elution Buffer (10 mM Tris-HCl, pH8.5 at 25ºC) is added into the center of the GD Column matrix and is completely absorbed. [3]Using TE (10 mM Tris-HCl, 1 mM EDTA, pH8.0) for elution is beneficial as EDTA preserves DNA for long term storage. However, EDTA will affect PCR and other sensitive downstream applications. [4]If using water for elution, ensure the water pH is between 7.0 and 8.5. ddH_2O should be fresh as ambient CO_2 can quickly cause acidification. DNA Eluted in water should be stored at -20ºC to avoid degradation.

Troubleshooting

Problem	Possible Reasons / Solution
Low Yield	**Incomplete buffer preparation** Add 1 ml of Elution Buffer to GM1 Powder then vortex to ensure GM1 Powder is completely dissolved to obtain a working solution of 1 µg/µl. Check the box on the bottle label showing Elution Buffer is added. Once GM1 Powder is dissolved completely, centrifuge for a few seconds to spin down the mixture. Divide the GM1 Buffer into convenient volumes in several RNase-free 1.5 ml microcentrifuge tubes. The GM1 Buffer should be stored at -20ºC. Do not freeze and thaw GM1 Buffer more than 3 times. Add dd$H_2$0 (pH7.0-8.5) to Proteinase K (see the bottle label for volume) then vortex to ensure Proteinase K is completely dissolved. Check the box on the bottle showing dd$H_2$0 is added. Once it is dissolved completely, centrifuge for a few seconds to spin down the mixture. For extended periods, store the dd$H_2$0 and Proteinase K mixture at 4ºC. Use only fresh dd$H_2$0 as ambient CO_2 can quickly cause acidification. Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle showing absolute ethanol is added. Be sure and close the bottle tightly after each use to avoid ethanol evaporation. **Incorrect lysis** Be sure the dried blood spot sample is completely immersed in the buffer. Make sure 1 µl of GM1 Buffer is added to 200 µl of GM2 Buffer. To increase DNA yield, 20 µl of 1M DTT shall be added to male urine samples which contain sperm cells. **Incorrect DNA elution step** Ensure that Elution Buffer, TE or water is added into the CENTER of the GD Column matrix and is completely absorbed. Use pre-heated Elution Buffer, TE, or water (60~70ºC). If using water for elution, ensure the water pH is between 7.0 and 8.5. ddH_2O should be fresh as ambient CO_2 can quickly cause acidification.
Eluted DNA Does Not Perform Well In Downstream Applications	**Residual Ethanol Contamination** Following the wash step, dry the GD Column with additional centrifugation at 14,000-16,000 x g for 5 minutes to ensure the GD Column membrane is completely dry.