ImaSpin® Genomic DNA Extraction Kit –Blood & Cell & Bacteria

For purification of genomic DNA from whole blood, buffy coat, lymphocytes, body fluids, cultured cells, and bacteria

Precautions

I. Handling Requirements
When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles.

II. Equipment and Reagents to Be Supplied by User

- Ethanol (96–100%)*
- 1.5 ml microcentrifuge tubes
- Pipet tips with aerosol barrier
- Vortexer
- Microcentrifuge (with rotor for 1.5 ml tubes) may be required for some samples

* Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone.

III. Waste Handling

- Treat waste with the country, federal, state and local regulations.

IV. Important points before use

- Do not use the product if it has expired.
- Add absolute ethanol (see the bottle label for volume) to IW2 Buffer then mix by shaking for a few seconds and tick the checkbox of the label on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.

Kit Contents

RBC Lysis Buffer
IGT Buffer
IGB Buffer
Binding Buffer
IW1 Buffer
IW2 Buffer (Add Ethanol)
Elution Buffer
Proteinase K (Add PK storage Buffer)
PK storage Buffer
IG Column

Storage and Stability:

1. This kit should be stored at room temperature.
2. Proteinase K should be stored at 4 °C upon arrival.

Description

ImaSpin® Genomic DNA Extraction Kit –Blood & Cell & Bacteria is designed by patented technology for purification of total DNA (including genomic, mitochondrial and viral DNA) from whole blood, plasma, serum, buffy coat, bacteria, up to $1x10^7$ cultured cells. The protocol uses buffer contains chaotropic salt to lyse cells and degrade protein. DNA will bind to special column. After washing off the contaminants, the

purified DNA is eluted by low salt elution buffer. Purified DNA is suitable for PCR or other enzymatic reactions.

ImaSpin® Genomic DNA Extraction Kit –Blood & Cell & Bacteria Test Data

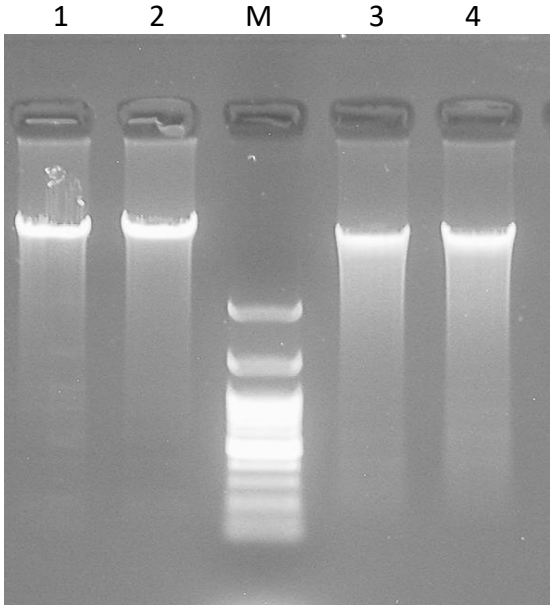

Fig 1. Whole Blood Genomic DNA extraction Comparison

Genomic DNA from 200 µl whole blood samples was extracted using the ImaSpin® Genomic DNA Extraction Kit –Blood & Cell & Bacteria and competitive brand Q. 10 µl from 100 µl eluates of purified genomic DNA was analyzed by electrophoresis on a 1 % agarose gel.

1-2 = ImaSpin® Genomic DNA Extraction Kit –Blood & Cell & Bacteria

3-4 = Competitive brand Q

M = 1 Kb DNA Ladder

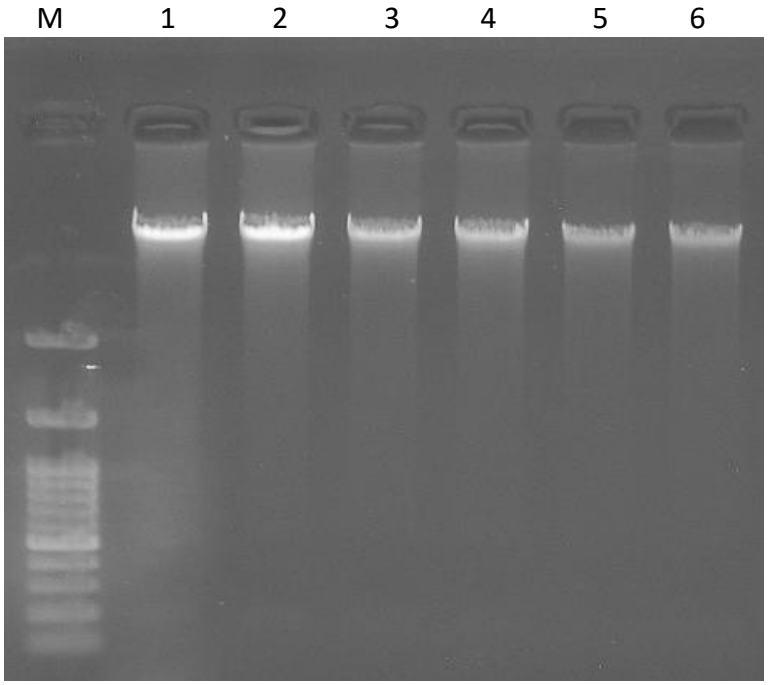

Fig 2. Whole Blood Genomic DNA extraction Stability test

Genomic DNA from 200, 100 and 50 µl whole blood samples was extracted using the ImaSpin® Genomic DNA Extraction Kit –Blood & Cell & Bacteria. 10 µl from 100 µl eluates of purified genomic DNA was analyzed by electrophoresis on a 1% agarose gel.

1-2 = 200 µl whole blood sample
3-4 = 100 µl whole blood sample
5-6 = 50 µl whole blood sample, M = 1 Kb DNA Ladder

Preparation before using
Add 1.1 ml of PK storage Buffer to the Proteinase K tube and mix by vortexing.
Store prepared Proteinase K (10 mg/ml) at 4 °C.

Fresh Blood and body fluids Protocol Procedure

1. 200 µl of Blood or Body Fluids add 20 µl of Proteinase K (10 mg/ml), mix by vortexing and incubate at 56 °C for 5 minutes.
   Optional RNA Removal Step
   If RNA-free genomic DNA is required, perform this optional step.
   Following 56 °C incubation, add 5 µl of RNase A (10 mg/ml) (not provided) to sample lysate and vortex to mix. Incubate at room temperature for 5 minutes.

2. Add 200 µl of IGB Buffer, mix by vortexing and incubate at 56 °C for 5 minutes.
   NOTE: Inverting the sample occasionally during incubation will facilitate Proteinase K digestion and cell lysis. Using an auto shaking system is more convenient when incubating samples.
3. At this time, preheat required Elution Buffer (100 µl per sample) in 56 °C (For DNA Elution Step).
4. Add 200 µl of Binding Buffer to the sample lysate and vortex immediately for 10 seconds to mix sample. If precipitate appears, break up by pipetting
   NOTE: It is important that the lysate and Binding Buffer are mixed thoroughly to yield a homogeneous solution.
5. Place an IG Column in a 2 ml Collection Tube. Transfer all of the mixture (including any insoluble precipitate) to the IG Column.
6. Centrifuge at 13,000 rpm (10,000 x g) for 3 minutes. Following centrifugation, if the mixture did not flow through the IG Column membrane, increase the centrifuge time until it passes completely.
7. Discard the flow-through. Place the IG Column back in the 2 ml Collection Tube.
8. Add 600 µl of IW1 Buffer to the IG Column.
9. Centrifuge at 13,000 rpm (10,000 x g) for 1 minute, then discard the flow-through. Place the IG Column back in the 2 ml Collection Tube.
10. Add 600 µl of IW2 Buffer (make sure absolute ethanol was added) to the IG Column.
11. Centrifuge at 13,000 rpm (10,000 x g) for 1 minute, then discard the flow-through. Place the IG Column back in the 2 ml Collection Tube.
12. Centrifuge for 3 minutes at 13,000 rpm (10,000 x g) to dry the column matrix.
13. Transfer the dried IG Column to a clean 1.5 ml microcentrifuge tube.
14. Add 100 µl of pre-heated Elution Buffer, TE Buffer or water into the CENTER of the column matrix.
    NOTE: Standard elution volume is 100 µl. If less sample to be used, reduce the elution volume (30-50 µl) to increase DNA concentration. If higher DNA yield required, repeat the DNA Elution Step to increase DNA recovery.
15. Let stand for at least 2 minutes to allow Elution Buffer, TE Buffer or water to be completely absorbed.
16. Centrifuge at 13,000 rpm (10,000 x g) for 2 minutes to elute the purified DNA.

Blood-Buffy Coat Preparation by RBC Lysis Protocol Procedure

1. Take 200 µl of whole blood into 2 ml microcentrifuge tube.
2. Add 0.6 ml of RBC Lysis Buffer and mix together by inverting the tube.
3. Shake the mixture at 100 rpm for 5 minutes.
4. Centrifuge the mixture at 2,500rpm (500 x g) for 5 minutes.
5. Discard supernatant.
6. Repeat step 2 ~ step 5 to wash the sample again.
7. Add 200 µl of PBS Buffer to resuspend the pellet
8. Add 20 µl of proteinase K, mix by vortexing and incubate at 56 °C for 5 minutes.
   Optional RNA Removal Step
   If RNA-free genomic DNA is required, perform this optional step.
   Following 56 °C incubation, add 5 µl of RNase A (10 mg/ml) (not provided) to sample lysate and vortex to mix. Incubate at room temperature for 5 minutes.

9. Add 200 µl of IGB Buffer, mix by vortexing and incubate at 56 °C for 5 minutes.
   NOTE: Inverting the sample occasionally during incubation will facilitate Proteinase K digestion and cell lysis. Using an auto shaking system is more convenient when incubating samples.
10. At this time, preheat required Elution Buffer (100 µl per sample) in 56 °C (For DNA Elution Step).
11. Add 200 µl of Binding Buffer to the sample lysate and vortex immediately for 10 seconds to mix sample. If precipitate appears, break up by pipetting
    NOTE: It is important that the lysate and Binding Buffer are mixed thoroughly to yield a homogeneous solution.
12. Place an IG Column in a 2 ml Collection Tube. Transfer all of the mixture (including any insoluble precipitate) to the IG Column.
13. Centrifuge at 13,000 rpm (10,000 x g) for 3 minutes. Following centrifugation, if the mixture did not flow through the IG Column membrane, increase the centrifuge time until it passes completely.
14. Discard the flow-through. Place the IG Column back in the 2 ml Collection Tube.
15. Add 600 µl of IW1 Buffer to the IG Column.
16. Centrifuge at 13,000 rpm (10,000 x g) for 1 minute, then discard the flow-through. Place the IG Column back in the 2 ml Collection Tube.
17. Add 600 µl of IW2 Buffer (make sure absolute ethanol was added) to the IG Column.
18. Centrifuge at 13,000 rpm (10,000 x g) for 1 minute, then discard the flow-through. Place the IG Column back in the 2 ml Collection Tube.
19. Centrifuge for 3 minutes at 13,000 rpm (10,000 x g) to dry the column matrix.
20. Transfer the dried IG Column to a clean 1.5 ml microcentrifuge tube.
21. Add 100 µl of pre-heated Elution Buffer, TE Buffer or water into the CENTER of the column matrix.
    NOTE: Standard elution volume is 100 µl. If less sample to be used, reduce the elution volume (30-50 µl) to increase DNA concentration. If higher DNA yield required, repeat the DNA Elution Step to increase DNA recovery.
22. Let stand for at least 2 minutes to allow Elution Buffer, TE Buffer or water to be completely absorbed.
23. Centrifuge at 13,000 rpm (10,000 x g) for 2 minutes to elute the purified DNA.

Blood-Buffy Coat Preparation by Centrifugation Protocol Procedure

1. Take 2 - 5 ml of whole blood sample and centrifuge at 2,500rpm (500 x g) rpm for 10 minutes.
2. Use plastic dropper to take white buffy coat layer in the middle of whole blood sample and move the buffy coat into a new microcentrifuge tube.
3. Take 40 - 50 µl of buffy coat sample into a 1.5 ml centrifuge tube and add PBS until 200 µl then add 20 µl of proteinase K, mix by vortexing and incubate at 56 °C for 5 minutes.
   Optional RNA Removal Step
   If RNA-free genomic DNA is required, perform this optional step.
   Following 56 °C incubation, add 5 µl of RNase A (10 mg/ml) (not provided) to sample lysate and vortex to mix. Incubate at room temperature for 5 minutes.

4. Add 200 µl of IGB Buffer, mix by vortexing and incubate at 56 °C for 5 minutes.
   NOTE: Inverting the sample occasionally during incubation will facilitate Proteinase K digestion and cell lysis. Using an auto shaking system is more convenient when incubating samples.
5. At this time, preheat required Elution Buffer (100 µl per sample) in 56 °C (For DNA Elution Step).
6. Add 200 µl of Binding Buffer to the sample lysate and vortex immediately for 10 seconds to mix sample. If precipitate appears, break up by pipetting
   NOTE: It is important that the lysate and Binding Buffer are mixed thoroughly to yield a homogeneous solution.
7. Place an IG Column in a 2 ml Collection Tube. Transfer all of the mixture (including any insoluble precipitate) to the IG Column.
8. Centrifuge at 13,000 rpm (10,000 x g) for 3 minutes. Following centrifugation, if the mixture did not flow through the IG Column membrane, increase the centrifuge time until it passes completely.
9. Discard the flow-through. Place the IG Column back in the 2 ml Collection Tube.
10. Add 600 µl of IW1 Buffer to the IG Column.
11. Centrifuge at 13,000 rpm (10,000 x g) for 1 minute, then discard the flow-through. Place the IG Column back in the 2 ml Collection Tube.
12. Add 600 µl of IW2 Buffer (make sure absolute ethanol was added) to the IG Column.
13. Centrifuge at 13,000 rpm (10,000 x g) for 1 minute, then discard the flow-through. Place the IG Column back in the 2 ml Collection Tube.
14. Centrifuge for 3 minutes at 13,000 rpm (10,000 x g) to dry the column matrix.
15. Transfer the dried IG Column to a clean 1.5 ml microcentrifuge tube.
16. Add 100 µl of pre-heated Elution Buffer, TE Buffer or water into the CENTER of the column matrix.
    NOTE: Standard elution volume is 100 µl. If less sample to be used, reduce the elution volume (30-50 µl) to increase DNA concentration. If higher DNA yield required, repeat the DNA Elution Step to increase DNA recovery.
17. Let stand for at least 2 minutes to allow Elution Buffer, TE Buffer or water to be completely absorbed.
18. Centrifuge at 13,000 rpm (10,000 x g) for 2 minutes to elute the purified DNA.

Cultured Cell Protocol Procedure

1. Harvest cells according to step I. (for cells grown in suspension) or II & III. (for cells grown in a monolayer).
   I. Cells grown in suspension (do not use more than 1 x 10^7 cells with a normal set of chromosomes): Determine the number of cells. Centrifuge the appropriate number of cells for 5 minutes a 2,500rpm (500 x g) in a 1.5 ml microcentrifuge tube. Remove the supernatant completely and discard, taking care not to disturb the cell pellet. Continue with step 2.
   II. Cells grown in a monolayer (do not use more than 1 x 10^7 cells with a normal set of chromosomes): Cells grown in a monolayer can be detached from the culture flask by either trypsinization or using a cell scraper. To trypsinize cells: Determine the number of cells. Aspirate the medium and wash cells with PBS. Aspirate the PBS, and add 0.10–0.25 % trypsin. After cells have detached from the dish or flask, collect them in medium, and transfer the appropriate number of cells (do not use more than 1 x 10^7 cells with a normal set of chromosomes) to a 1.5 ml microcentrifuge tube. Centrifuge for 5 minutes at 2,500rpm (500 x g). Remove the supernatant completely and discard, taking care not to disturb the cell pellet. Continue with step 2.
   III. Using a cell scraper: Detach cells from the dish or flask. Transfer the appropriate number of cells (do not use more than 1 x 10^7 cells with a normal set of chromosomes) to a 1.5 ml microcentrifuge tube and centrifuge for 5 minutes at 300 x g. Remove the supernatant completely and discard, taking care not to disturb the cell pellet. Continue with step 2.
2. Resuspend cell pellet in PBS Buffer to a final volume of 200 µl.
3. Add 20 µl of proteinase K, mix by vortexing and incubate at 56 °C for 5 minutes.
   Optional RNA Removal Step
   If RNA-free genomic DNA is required, perform this optional step.
   Following 56 °C incubation, add 5 µl of RNase A (10 mg/ml) (not provided) to sample lysate and vortex to mix. Incubate at room temperature for 5 minutes.
4. Add 200 µl of IGB Buffer, mix by vortexing and incubate at 56 °C for 5 minutes.
   NOTE: Inverting the sample occasionally during incubation will facilitate Proteinase K digestion and cell lysis. Using an auto shaking system is more convenient when incubating samples.
5. At this time, preheat required Elution Buffer (100 µl per sample) in 56 °C (For DNA Elution Step).
6. Add 200 µl of Binding Buffer to the sample lysate and vortex immediately for 10 seconds to mix sample. If precipitate appears, break up by pipetting
   NOTE: It is important that the lysate and Binding Buffer are mixed thoroughly to yield a homogeneous solution.
7. Place an IG Column in a 2 ml Collection Tube. Transfer all of the mixture (including any insoluble precipitate) to the IG Column.
8. Centrifuge at 13,000 rpm (10,000 x g) for 3 minutes. Following centrifugation, if the mixture did not flow through the IG Column membrane, increase the centrifuge time until it passes completely.
9. Discard the flow-through. Place the IG Column back in the 2 ml Collection Tube.
10. Add 600 µl of IW1 Buffer to the IG Column.
11. Centrifuge at 13,000 rpm (10,000 x g) for 1 minute, then discard the flow-through. Place the IG Column back in the 2 ml Collection Tube.
12. Add 600 µl of IW2 Buffer (make sure absolute ethanol was added) to the IG Column.
13. Centrifuge at 13,000 rpm (10,000 x g) for 1 minute, then discard the flow-through. Place the IG Column back in the 2 ml Collection Tube.
14. Centrifuge for 3 minutes at 13,000 rpm (10,000 x g) to dry the column matrix.
15. Transfer the dried IG Column to a clean 1.5 ml microcentrifuge tube.
16. Add 100 µl of pre-heated Elution Buffer, TE Buffer or water into the CENTER of the column matrix.

NOTE: Standard elution volume is 100 µl. If less sample to be used, reduce the elution volume (30-50 µl) to increase DNA concentration. If higher DNA yield required, repeat the DNA Elution Step to increase DNA recovery.

17. Let stand for at least 2 minutes to allow Elution Buffer, TE Buffer or water to be completely absorbed.
18. Centrifuge at 13,000 rpm (10,000 x g) for 2 minutes to elute the purified DNA.

Gram-Negative Bacteria Protocol Procedure

1. Harvest cells (maximum 2 x 10^9 cells) in a microcentrifuge tube by centrifuging for 10 min at 5000 x g (7500 rpm). Discard supernatant.
2. Resuspend cell pellet in 200 µl of IGT Buffer.
3. Add 20 µl of proteinase K, mix by vortexing and incubate at 56 °C for 5 minutes.
   Optional RNA Removal Step
   If RNA-free genomic DNA is required, perform this optional step.
   Following 56 °C incubation, add 5 µl of RNase A (10 mg/ml) (not provided) to sample lysate and vortex to mix. Incubate at room temperature for 5 minutes.
4. Add 200 µl of IGB Buffer, mix by vortexing and incubate at 56 °C for 10 minutes.
   NOTE: Inverting the sample occasionally during incubation will facilitate Proteinase K digestion and cell lysis. Using an auto shaking system is more convenient when incubating samples.
5. At this time, preheat required Elution Buffer (100 µl per sample) in 56 °C (For DNA Elution Step).
6. Add 200 µl of Binding Buffer to the sample lysate and vortex immediately for 10 seconds to mix sample. If precipitate appears, break up by pipetting
   NOTE: It is important that the lysate and Binding Buffer are mixed thoroughly to yield a homogeneous solution.
7. Place an IG Column in a 2 ml Collection Tube. Transfer all of the mixture (including any insoluble precipitate) to the IG Column.
8. Centrifuge at 13,000 rpm (10,000 x g) for 3 minutes. Following centrifugation, if the mixture did not flow through the IG Column membrane, increase the centrifuge time until it passes completely.
9. Discard the flow-through. Place the IG Column back in the 2 ml Collection Tube.
10. Add 600 µl of IW1 Buffer to the IG Column.
11. Centrifuge at 13,000 rpm (10,000 x g) for 1 minute, then discard the flow-through. Place the IG Column back in the 2 ml Collection Tube.
12. Add 600 µl of IW2 Buffer (make sure absolute ethanol was added) to the IG Column.
13. Centrifuge at 13,000 rpm (10,000 x g) for 1 minute, then discard the flow-through. Place the IG Column back in the 2 ml Collection Tube.
14. Centrifuge for 3 minutes at 13,000 rpm (10,000 x g) to dry the column matrix.
15. Transfer the dried IG Column to a clean 1.5 ml microcentrifuge tube.
16. Add 100 µl of pre-heated Elution Buffer, TE Buffer or water into the CENTER of the column matrix.
    NOTE: Standard elution volume is 100 µl. If less sample to be used, reduce the elution volume (30-50 µl) to increase DNA concentration. If higher DNA yield required, repeat the DNA Elution Step to increase DNA recovery.
17. Let stand for at least 2 minutes to allow Elution Buffer, TE Buffer or water to be completely absorbed.
18. Centrifuge at 13,000 rpm (10,000 x g) for 2 minutes to elute the purified DNA.

Gram-Positive Bacteria Protocol Procedure

Prepare **Lysozyme Buffer** (Not provided in the Kit): (20mg/ml lysozyme; 20mM Tris-HCl pH 8.0; 2mM EDTA; 1% Triton X-100), prepare the Lysozyme Buffer fresh immediately prior to use.

1. Harvest cells (maximum 2 x 10^9 cells) in a microcentrifuge tube by centrifuging for 10 min at 5000 x g (7500 rpm). Discard supernatant.
2. Resuspend cell pellet in 200 µl of **Lysozyme Buffer**.
3. Incubate for at least 30 min at 37°C, invert tube every 5 minutes
4. Add 20 µl of proteinase K, mix by vortexing and incubate at 56 °C for 5 minutes.
   Optional RNA Removal Step
   If RNA-free genomic DNA is required, perform this optional step.
   Following 56 °C incubation, add 5 µl of RNase A (10 mg/ml) (not provided) to sample lysate and vortex to mix. Incubate at room temperature for 5 minutes.

5. Add 200 µl of IGB Buffer, mix by vortexing and incubate at 56 °C for 30 minutes.
   NOTE: Inverting the sample occasionally during incubation will facilitate Proteinase K digestion and cell lysis. Using an auto shaking system is more convenient when incubating samples.
6. At this time, preheat required Elution Buffer (100 µl per sample) in 56 °C (For DNA Elution Step).
7. Add 200 µl of Binding Buffer to the sample lysate and vortex immediately for 10 seconds to mix sample. If precipitate appears, break up by pipetting
   NOTE: It is important that the lysate and Binding Buffer are mixed thoroughly to yield a homogeneous solution.
8. Place an IG Column in a 2 ml Collection Tube. Transfer all of the mixture (including any insoluble precipitate) to the IG Column.
9. Centrifuge at 13,000 rpm (10,000 x g) for 3 minutes. Following centrifugation, if the mixture did not flow through the IG Column membrane, increase the centrifuge time until it passes completely.
10. Discard the flow-through. Place the IG Column back in the 2 ml Collection Tube.
11. Add 600 µl of IW1 Buffer to the IG Column.
12. Centrifuge at 13,000 rpm (10,000 x g) for 1 minute, then discard the flow-through. Place the IG Column back in the 2 ml Collection Tube.
13. Add 600 µl of IW2 Buffer (make sure absolute ethanol was added) to the IG Column.
14. Centrifuge at 13,000 rpm (10,000 x g) for 1 minute, then discard the flow-through. Place the IG Column back in the 2 ml Collection Tube.
15. Centrifuge for 3 minutes at 13,000 rpm (10,000 x g) to dry the column matrix.
16. Transfer the dried IG Column to a clean 1.5 ml microcentrifuge tube.
17. Add 100 µl of pre-heated Elution Buffer, TE Buffer or water into the CENTER of the column matrix.
    NOTE: Standard elution volume is 100 µl. If less sample to be used, reduce the elution volume (30-50 µl) to increase DNA concentration. If higher DNA yield required, repeat the DNA Elution Step to increase DNA recovery.
18. Let stand for at least 2 minutes to allow Elution Buffer, TE Buffer or water to be completely absorbed.
19. Centrifuge at 13,000 rpm (10,000 x g) for 2 minutes to elute the purified DNA.

Troubleshooting

Problem	Possible Reasons / Solution
Low Yield	➢ Ensure absolute ethanol was added to IW2 Buffer and close the bottle tightly after each use to avoid ethanol evaporation. ➢ Reduce the sample material. ➢ Following Binding Buffer addition to the lysate, break up any precipitate as much as possible prior to loading to IG Column. ➢ Ensure Elution Buffer or water is added into the CENTER of the column matrix. ➢ Elute twice to increase yield.
Eluted DNA does not perform well in downstream applications	➢ Following the Wash Step, dry the IG column by incubate at 60 °C for 5 minutes. ➢ Use fresh blood, long term storage ample may result in fragmentation of genomic DNA. ➢ 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. ➢ If using water for elution, ensure the water pH is between 7.5 and 8.5 ddH_2O should be fresh as ambient CO_2 can quickly cause acidification. DNA eluted in water should be stored at -20 °C to avoid degradation