ImaSpin® Genomic DNA Extraction Kit –Tissue & Swab & Forensic samples

For extraction of genomic DNA from a variety to animal tissues, blood spots, feed-soil Sample**,** cigarette butts, hair roots, chewing gum**,** buccal swabs, betel nut residue, stool sample, saliva, sputum specimens, and cultured yeast.

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

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
2 ml Collection Tubes

Storage and Stability:

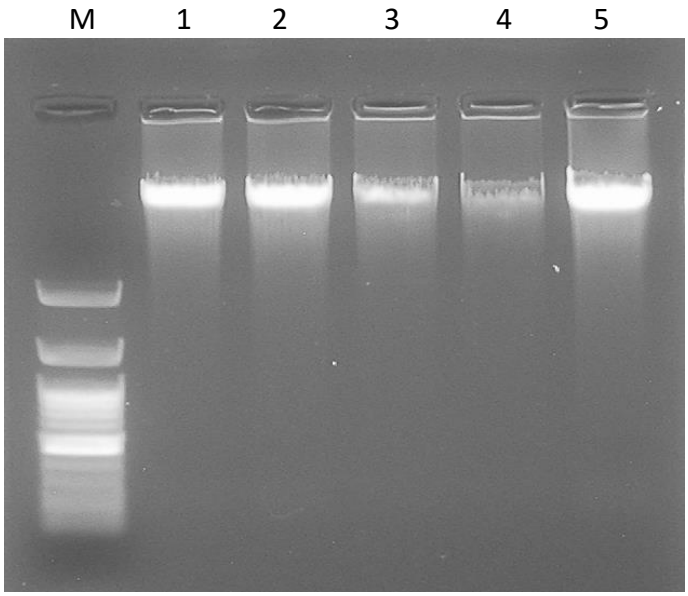
1. This kit should be stored at room temperature.
2. Proteinase K should be stored at 4 °C upon arrival.

Description

ImaSpin® Genomic DNA Extraction Kit –Tissue & Swab & Forensic samples is designed by patented technology for purification of total DNA (including genomic, mitochondrial and viral DNA) from a variety to animal tissues, paraffin-embedded tissue, swab, blood spots, forensic specimens and cultured yeast.

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. The purified genomic DNA can be directly used for downstream applications, such as PCR, AFLP/PADP, RFLP, Real-time PCR, screening, restriction enzyme digestion, southern blotting...etc.

ImaSpin® Genomic DNA Extraction Kit –Tissue & Swab & Forensic samples Test Data



Genomic DNA from a variety of tissue samples was extracted using the ImaSpin® Genomic DNA Extraction Kit –Tissue & Swab & Forensic samples. The purified genomic DNA was analyzed by electrophoresis on a 1 % agarose gel.
M = 1 Kb DNA Ladder
1 = Rat tail 0.5 cm
2 = Mouse liver 10 mg
3 = Mouse spleen 10 mg
4 = Mouse heart 10 mg
5 = Mouse Kidney 10 mg

Preparation before using

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

Tissue Protocol Procedure

1. Cut the solid tissue to small pieces (up to 25 mg) (or 0.5 cm of mouse tail) and transfer to a microcentrifuge tube (not provided). If tissue has a higher number of cells (e.g. spleen or liver), reduce starting material to 10mg.
2. Add 200 µl of IGT Buffer and 20 µl of Proteinase K (10 mg/ml) to the tube and mix by vortexing.
3. Incubate at 56 °C overnight or until the sample has been completely lysed.
   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. If insoluble material remains following incubation, centrifuge for 2 minutes a 13,000 rpm (10,000 x g) then carefully transfer the supernatant to a new 1.5 ml microcentrifuge tube.
5. 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.
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.

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 5 x 10^6 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 5 x 10^6 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 5 x 10^6 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 5 x 10^6 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 IGT 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.

Amniotic Fluid Protocol Procedure

1. Transfer up to 15 ml of amniotic fluid to a 15 ml centrifuge tube. Centrifuge for 3 minutes at 14-16,000 x g then discard the supernatant.
2. Add 200 µl of IGT Buffer to resuspend the pellet and transfer the mixture to a 1.5 ml microcentrifuge tube. Add 20 µl of Proteinase K to the sample mixture and shake vigorously. Incubate at 56 °C for 30 minutes. During incubation, invert the tube every 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.

3. 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.
4. At this time, preheat required Elution Buffer (100 µl per sample) in 56 °C (For DNA Elution Step).
5. 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.
6. Place an IG Column in a 2 ml Collection Tube. Transfer all of the mixture (including any insoluble precipitate) to the IG Column.
7. 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.
8. Discard the flow-through. Place the IG Column back in the 2 ml Collection Tube.
9. Add 600 µl of IW1 Buffer to the IG Column.
10. 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.
11. Add 600 µl of IW2 Buffer (make sure absolute ethanol was added) 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. Centrifuge for 3 minutes at 13,000 rpm (10,000 x g) to dry the column matrix.
14. Transfer the dried IG Column to a clean 1.5 ml microcentrifuge tube.
15. 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.
16. Let stand for at least 2 minutes to allow Elution Buffer, TE Buffer or water to be completely absorbed.
17. Centrifuge at 13,000 rpm (10,000 x g) for 2 minutes to elute the purified DNA.

Hair roots Protocol Procedure

1. Cut the hair roots into 0.5 - 1 cm pieces.
2. Transfer the pieces to a 1.5 ml microcentrifuge tube. Add 200 µl of IGT buffer and 20 µl of Proteinase K, close the lid, and mix for 10 seconds. Incubate at 56 °C for 1 hour to lyse the sample.
   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.
3. Briefly centrifuge the sample tube to remove drops from the inside of the lid.
4. Pipette 200 µl of clear sample solution to a new 1.5 ml RNase-free microcentrifuge tube.
5. 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.
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.

Insect Protocol Procedure

1. Transfer up to 50 mg of insect tissue to a mortar. Add liquid nitrogen to the mortar and grind the tissue thoroughly using a pestle. Refill the mortar occasionally with liquid nitrogen to keep the sample frozen.
2. Transfer the tissue powder to a 1.5 ml microcentrifuge tube.
3. Add 200 µl of IGT Buffer and 20 µl of Proteinase K then vortex thoroughly.
4. Incubate at 56 °C for 1-3 hours or until the sample lysate becomes clear.
   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. Briefly centrifuge the sample tube to remove drops from the inside of the lid.
6. Pipette 200 µl of clear sample solution to a new 1.5 ml RNase-free microcentrifuge tube.
7. 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.
8. At this time, preheat required Elution Buffer (100 µl per sample) in 56 °C (For DNA Elution Step).
9. 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.
10. Place an IG Column in a 2 ml Collection Tube. Transfer all of the mixture (including any insoluble precipitate) to the IG Column.
11. 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.
12. Discard the flow-through. Place the IG Column back in the 2 ml Collection Tube.
13. Add 600 µl of IW1 Buffer 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. Add 600 µl of IW2 Buffer (make sure absolute ethanol was added) 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. Centrifuge for 3 minutes at 13,000 rpm (10,000 x g) to dry the column matrix.
18. Transfer the dried IG Column to a clean 1.5 ml microcentrifuge tube.
19. 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.
20. Let stand for at least 2 minutes to allow Elution Buffer, TE Buffer or water to be completely absorbed.
21. 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 samples, 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