# NIGERIAN INSTITUTE OF MEDICAL RESEARCH

# DATA SHEET

# GENOMIC DNA Purification Kit

**For *in vitro* use only!**



**Shipping: shipped ambient temperature**

**Storage conditions: Store at room temperature (proteinase K can be refrigerated)**

**Shelf life: 12 months**

## Description of Kit:

This spin column based DNA purification kit is formulated for the rapid isolation of genomic DNA from whole blood, dried blood spot, bacteria cells, animal cells, and plant tissues. Kit yields up to 30µg DNA, and the extracted DNA is suitable for all downstream applications.

Spin column based genomic DNA extraction from whole blood, dried blood spot, bacteria (gram positive and gram negative), plant, insects, and animal tissue samples.

## Kit Content (for 50 preps kit):

30ml Lysis buffer

30ml Binding Buffer

12ml Wash Buffer 1 concentrate (add48 ml of absolute ethanol before use)

18ml Wash Buffer 2 concentrate (add 42ml of absolute ethanol before use)

10ml Elution Buffer

1ml Proteinase K

50 Spin Columns

50 collection tubes

## To be provided by the user

96 – 99% (Absolute) ethanol

1.5ml Microcentrifuge tube

RNAse A (optional)

**VERY IMPORTANT NOTE**

**PLEASE ADD**

- 48ml of absolute ethanol to the Wash Buffer 1 Concentrate
- 42ml of absolute ethanol to the Wash Buffer 2 Concentrate

## DNA PREPARATION FROM BLOOD

1. Add 100 µl of whole blood into a pre-labelled 1.5ml microcentrifuge tube
2. Add 300µl of Lysis buffer
3. Vortex vigorously for 30 seconds
4. Add 10µl of Proteinase K. Vortex again
5. Incubate at 60ºC for 10 minutes.
6. Allow to cool, and then add 300µl of binding buffer.
7. Vortex to mix, then centrifuge at 10,000 g for 1 minute.
8. Add 200µl of absolute ethanol to the sample
9. Place a spin column into a collection tube, then transfer sample directly into the middle of the spin column
10. Centrifuge at 10,000g for 1 minute
11. Discard the flow through, blot the collection tube on a tissue, and reinsert the spin column into the collection tube.
12. Add 500µl of wash buffer 1.
13. Centrifuge at 10,000g for 1 minute

14. Discard the flow through, blot the collection tube on a tissue, and reinsert the spin column into the collection tube.
15. Add 500µl of wash buffer 2
16. Centrifuge at 10,000g for 1 minute
17. Discard the flow through, blot the collection tube on a tissue, and reinsert the spin column into the collection tube.
18. Dry spin the spin column and the collection tube at highest speed for 2 minutes.
19. Discard the collection tube and insert the spin column into a new 1.5ml microcentrifuge tube
20. Add 50 - 100µl of elution buffer
21. Incubate at room temperature for 1 minute, then centrifuge at 10,000g for 1 minute.
22. Store the eluted DNA at -20ºC

## DNA PREPARATION FROM DRIED BLOOD SPOT

1. Add a disc of dried blood spot in a microentrifuge tube.
2. Add 300µl of Lysis buffer
3. Vortex vigorously for 30 seconds, and incubate at room temperature for 1 minute.
4. Add 10µl of Proteinase K. Vortex again
5. Incubate at 60ºC for 15 minutes.
6. Allow to cool, and then add 300µl of binding buffer.
7. Vortex to mix, then centrifuge at 10,000 g for 1 minute.
8. Add 200µl of absolute ethanol to the sample
9. Place a spin column into a collection tube, then transfer sample directly into the middle of the spin column
10. Centrifuge at 10,000g for 1 minute
11. Discard the flow through, blot the collection tube on a tissue, and reinsert the spin column into the collection tube.
12. Add 500µl of wash buffer 1.

13. Centrifuge at 10,000g for 1 minute
14. Discard the flow through, blot the collection tube on a tissue, and reinsert the spin column into the collection tube.
15. Add 500µl of wash buffer 2
16. Centrifuge at 10,000g for 1 minute
17. Discard the flow through, blot the collection tube on a tissue, and reinsert the spin column into the collection tube.
18. Dry spin the spin column and the collection tube at highest speed for 2 minutes.
19. Discard the collection tube and insert the spin column into a new 1.5ml microcentrifuge tube
20. Add 50 - 100µl of elution buffer
21. Incubate at room temperature for 1 minute, then centrifuge at 10,000g for 1 minute.
22. Store the eluted DNA at -20ºC

## DNA PREPARATION FROM BACTERIA

1. Harvest bacteria cells into 100µl of PBS
2. Vortex vigorously to mix

   If using bacteria cells in broth, centrifuge 1 ml of the bacterial mixture at 10,000g for 5 minutes, discard the supernatant. Continue from step 3.
3. Add 300µl of Lysis buffer
4. Vortex vigorously for 30 seconds
5. Add 10µl of Proteinase K. Vortex again.
6. Incubate at 60ºC for 10 minutes for gram negative bacteria or 15 minutes for gram positive bacteria
7. Allow to cool, and then add 300µl of binding buffer.
8. Vortex to mix, then centrifuge at 10,000 g for 1 minute.
9. Add 200µl of absolute ethanol to the sample
10. Place a spin column into a collection tube, then transfer sample directly into the middle of the spin column
11. Centrifuge at 10,000g for 1 minute

12. Discard the flow through, blot the collection tube on a tissue, and reinsert the spin column into the collection tube.
13. Add 500µl of wash buffer 1.
14. Centrifuge at 10,000g for 1 minute
15. Discard the flow through, blot the collection tube on a tissue, and reinsert the spin column into the collection tube.
16. Add 500µl of wash buffer 2
17. Centrifuge at 10,000g for 1 minute
18. Discard the flow through, blot the collection tube on a tissue, and reinsert the spin column into the collection tube.
19. Dry spin the spin column and the collection tube at highest speed for 2 minutes.
19. Discard the collection tube and insert the spin column into a new 1.5ml microcentrifuge tube
20. Add 50 - 100µl of elution buffer
21. Incubate at room temperature for 1 minute, then centrifuge at 10,000g for 1 minute.
22. Store the eluted DNA at -20ºC

## DNA PREPARATION FROM PLANTS, INSECTS, AND ANIMAL TISSUES

1. Weigh not more than 100mg of plant, insect or animal material
2. Homogenize with 1ml of PBS using a mortar and pestle (if you have a mini bead beater, you can homogenize in a cryovial tube with beads)
3. Transfer into a 1.5ml microcentrifuge tube and Centrifuge at 10,000g for 1 minute
4. Discard supernatant
5. Add 300µl of Lysis buffer to the tissue pellet
6. Vortex vigorously for 30 seconds
7. Add 10µl of Proteinase K. Vortex again.
8. Incubate at 60ºC for 20 minutes
9. Allow to cool, and then add 300µl of binding buffer.
10. Vortex to mix, then centrifuge at 10,000 g for 1 minute.

11. Add 200µl of absolute ethanol to the sample
12. Place a spin column into a collection tube, then transfer sample directly into the middle of the spin column
13. Centrifuge at 10,000g for 1 minute
14. Discard the flow through, blot the collection tube on a tissue, and reinsert the spin column into the collection tube.
15. Add 500µl of wash buffer 1.
16. Centrifuge at 10,000g for 1 minute
17. Discard the flow through, blot the collection tube on a tissue, and reinsert the spin column into the collection tube.
18. Add 500µl of wash buffer 2
19. Centrifuge at 10,000g for 1 minute
20. Discard the flow through, blot the collection tube on a tissue, and reinsert the spin column into the collection tube.
21. Dry spin the spin column and the collection tube at highest speed for 2 minutes.
22. Discard the collection tube and insert the spin column into a new 1.5ml microcentrifuge tube
23. Add 50 - 100µl of elution buffer
24. Incubate at room temperature for 1 minute, then centrifuge at 10,000g for 1 minute.
25. Store the eluted DNA at -20ºC

## DNA PREPARATION FROM FISH TISSUES

1. Weigh not more than 100mg of fish tissue into a 1.5ml microcentrifuge tube
2. Add 300µl of Lysis buffer
3. Vortex vigorously for 30 seconds, and incubate at room temperature for 1 minute.
5. Add 10µl of Proteinase K. Vortex again.
6. Incubate at 60ºC for 20 minutes
7. Allow to cool, and then add 300µl of binding buffer.

8. Vortex to mix, then centrifuge at 10,000 g for 1 minute.
9. Add 200µl of absolute ethanol to the sample
10. Place a spin column into a collection tube, then transfer sample directly into the middle of the spin column
11. Centrifuge at 10,000g for 1 minute
12. Discard the flow through, blot the collection tube on a tissue, and reinsert the spin column into the collection tube.
13. Add 500µl of wash buffer 1.
14. Centrifuge at 10,000g for 1 minute
15. Discard the flow through, blot the collection tube on a tissue, and reinsert the spin column into the collection tube.
16. Add 500µl of wash buffer 2
17. Centrifuge at 10,000g for 1 minute
18. Discard the flow through, blot the collection tube on a tissue, and reinsert the spin column into the collection tube.
19. Dry spin the spin column and the collection tube at highest speed for 2 minutes.
20. Discard the collection tube and insert the spin column into a new 1.5ml microcentrifuge tube
21. Add 50 - 100µl of elution buffer
22. Incubate at room temperature for 1 minute, then centrifuge at 10,000g for 1 minute.
23. Store the eluted DNA at -20ºC