ig® HMW DNA Extraction Kit

Intact Genomics®

Catalog #	Package Size
3812	50 Preps
3815	200 Preps

Introduction

Ig® HMW DNA Extraction Kit is designed for isolation of high-molecular-weight (HMW) DNA from white blood cells, plant tissue and Gram-positive and Gram-negative bacteria. DNA purified with this system is particularly suitable for long-read sequencing platforms.

Kit Components and Storage

The below reagents should be stored at –20°C.

- Proteinase K Solution (20mg/ml)
- RNase A solution (100 ng/ml)
- Lysozyme Solution (50 mg/ml)

The below reagents should be stored at 4°C.

- PBS
- HMW Blood Lysis Buffer
- HMW Lysis Buffer A
- Protein Precipitation Solution

Not included but required:

- TE

Key Benefits

- Optimized and quick protocol with no hazardous/toxic chemicals
- Simplified preparation steps for extracting high-purity HMW DNA upto 1 megabase
- Best for long-read sequencing

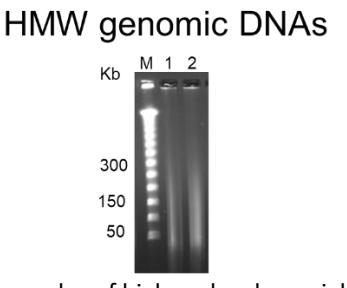

Figure 1. Examples of high molecular weight (HMW) genomic DNAs prepared with ig® HMW gDNA preparation kit. In example samples 1 & 2, the majority of HMW gDNA is >30 ~ 300 kb, some are up to megabase in size. M. Lambda ladder DNA marker.

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Protocol - Isolating HMW DNA from Whole Blood (Human)

1. To a 150ul blood sample add 450µl of HMW Blood Lysis Buffer in a 1.7ml microcentrifuge tube.

2. Invert the tube 5–6 times to mix.

3. Incubate the mixture for 10 minutes at room temperature (invert 2–3 times once during the incubation) to lyse the red blood cells. Centrifuge at 13,000× rpm for 20 seconds at room temperature.

4. Remove and discard as much supernatant as possible without disturbing the visible white pellet.

5. Repeat Steps 3–4.

6. Add 50µl of PBS to the cell pellet and vortex the tube vigorously until the white blood cells are resuspended (10–15 seconds).

7. Add 250µl of HMW Lysis Buffer A. Invert the tube 5 times to mix and lyse the white blood cells.

8. Add 3.0µl of RNase A Solution to the lysate and mix the sample by inverting the tube 5 times. Incubate the mixture at 37°C for 15 minutes.

9. Add 20µl of Proteinase K Solution to lysate and mix the sample by inverting the tube 5 times. Incubate the mixture at 56°C for 15 minutes. Cool to room temperature for at least 5 minutes.

10. Add 300µl of Protein Precipitation Solution (equal amount lysate) to the lysate. Invert the tube 10 times to mix. Incubate on ice for 5 minutes.

11. Centrifuge at 13,000 × rpm for 10 minutes at room temperature. A dark brown protein pellet should be visible.

12. Slowly transfer the supernatant (600µl) to a clean 1.7ml microcentrifuge tube by 200µl wide bore pipette tip the sample into a tube containing 600µl of room-temperature isopropanol.

13. Gently mix the solution by gently inverting the tube eight times. Allow 1 minute at room temperature and repeat the inversion. White thread-like strands of DNA may become visible. Centrifuge at 13,000 × rpm for 5 minutes at room temperature. The DNA will be visible as a small white pellet.

14. Carefully remove the supernatant by 1000µl pipette tip and add 1000µl of room temperature 70% ethanol to the DNA. Gently invert the tube several times to wash the DNA pellet and the sides of the microcentrifuge tube. Centrifuge at 13,000 × rpm for 5 minutes at room temperature.

15. Carefully remove the supernatant by 1000µl pipette tip. Invert the tube on clean absorbent paper and air-dry the pellet for 10–15 minutes.

16. Add 50µl of DNA TE Solution to the tube. Do not vortex because this will cause mechanical shearing and decrease average fragment size. Rehydrate the DNA by incubating the solution overnight at room temperature. Store the DNA at 2–8°C

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Protocol - Isolating HMW Genomic DNA from Plant Tissue (Maize, Rice, Wheat, etc.)

1. Process leaf tissue by freezing with liquid nitrogen and grinding into a fine powder using a pestle. Add 50mg of this leaf powder to a 1.7ml microcentrifuge tube.

2. Add 500µl of HMW Lysis Buffer A, and vortex 1–5 seconds to wet the tissue.

3. Incubate at 65°C for 30 minutes.

4. Cool the lysate to room temperature for 5 minutes. Add 3µl of RNase A Solution to the sample and mix by inverting the tube 5 times. Incubate the mixture at 37°C for 15 minutes.

5. Add 20µl of Proteinase K Solution to lysate and mix the sample by inverting the tube 5 times. Incubate the mixture at 56°C for 15 minutes. Cool to room temperature for at least 5 minutes.

6. Centrifuge at 13,000× rpm for 3 minutes at room temperature to pellet any insoluble material. Transfer the lysate (400 µl) to a clean 1.7ml microcentrifuge tube.

7. Add 400µl of Protein Precipitation Solution (equal amount lysate) to the lysate. Invert the tube 10 times to mix. Incubate on ice for 5 minutes.

8. Centrifuge at 13,000 × rpm for 10 minutes at room temperature. A dark brown protein pellet should be visible.

9. Slowly transfer the supernatant (600µl) to a clean 1.7ml microcentrifuge tube by 200µl wide bore pipette tip the sample into a tube containing 600µl of room-temperature isopropanol.

10. Gently mix the solution by gently inverting the tube eight times. Allow 1 minute at room temperature and repeat the inversion. White thread-like strands of DNA may become visible. Centrifuge at 13,000 × rpm for 5 minutes at room temperature. The DNA will be visible as a small white pellet.

11. Carefully remove the supernatant by 1000µl pipette tip and add 1000µl of room temperature 70% ethanol to the DNA. Gently invert the tube several times to wash the DNA pellet and the sides of the microcentrifuge tube. Centrifuge at 13,000 × rpm for 5 minutes at room temperature.

12. Carefully remove the supernatant by 1000µl pipette tip. Invert the tube on clean absorbent paper and air-dry the pellet for 10–15 minutes.

13. Add 50µl of DNA TE Solution to the tube. Do not vortex because this will cause mechanical shearing and decrease average fragment size. Rehydrate the DNA by incubating the solution overnight at room temperature. Store the DNA at 2–8°C

Protocol - Isolating HMW DNA from Gram-Positive and Gram-Negative Bacteria

1. Add 1ml of an overnight culture to a 1.5ml microcentrifuge tube.

2. Centrifuge at 13,000× rpm for 2 minutes to pellet the cells. Remove the supernatant and resuspend the cells thoroughly in 100µl of PBS.

3. Add 500µl of HMW Lysis Buffer A and 10µl of 50mg/ml lysozyme. Using 1,000µl pipette tips, mix the solution five times to lyse the cells. Incubate the sample at 37°C for 30 minutes.

4. Add 3.0µl of RNase A Solution to the lysate and mix the sample by inverting the tube 5 times. Incubate the mixture at 37°C for 15 minutes.

5. Add 20µl of Proteinase K Solution to lysate and mix the sample by inverting the tube 5 times. Incubate the mixture at 56°C for 15 minutes. Cool to room temperature for at least 5 minutes.

6. Add 600µl of Protein Precipitation Solution (equal amount lysate) to the lysate. Invert the tube 10 times to mix. Incubate on ice for 5 minutes.

7. Centrifuge at 13,000 × rpm for 10 minutes at room temperature. A dark brown protein pellet should be visible.

8. Slowly transfer the supernatant (600µl) to a clean 1.7ml microcentrifuge tube by 200µl wide bore pipette tip the sample into a tube containing 600µl of room-temperature isopropanol.

9. Gently mix the solution by gently inverting the tube eight times. Allow 1 minute at room temperature and repeat the inversion. White thread-like strands of DNA may become visible. Centrifuge at 13,000 × rpm for 5 minutes at room temperature. The DNA will be visible as a small white pellet.

10. Carefully remove the supernatant by 1000µl pipette tip and add 1000µl of room temperature 70% ethanol to the DNA. Gently invert the tube several times to wash the DNA pellet and the sides of the microcentrifuge tube. Centrifuge at 13,000 × rpm for 5 minutes at room temperature.

11. Carefully remove the supernatant by 1000µl pipette tip. Invert the tube on clean absorbent paper and air-dry the pellet for 10–15 minutes.

12. Add 50µl of DNA TE Solution to the tube. Do not vortex because this will cause mechanical shearing and decrease average fragment size. Rehydrate the DNA by incubating the solution overnight at room temperature. Store the DNA at 2–8°C.

References

1. Miller, S.A., Dykes, D.D. and Polesky, H.F. (1988) A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. 16, 1215.

2. Beutler, E., Gelbart, T. and Kuhl, W. (1990) Interference of heparin with the polymerase chain reaction. BioTechniques 9, 166.

3. U.S. Department of Labor, Occupational Safety and Health Administration (1991) Occupational exposure to bloodborne pathogens, final rule. Federal Register 56, 64175.