Kit Manual (19.08.2010)

*m*i-Blood Genomic DNA HTP Prep Kit

Cat. no:
mi-BGHTP10 [10x 96 preparations]
mi-BGHTP30 [30x 96 preparations]

(mi-BGHTP3 [3x 96 preparations] Testkit)

This kit is for research purposes only.
Not for use in diagnostic analysis.
For *in vitro* use only.

A. Introduction

The metaBIOn Blood Genomic DNA HTP Prep Kit is suitable for preparation of pure genomic DNA from small-scale whole blood, serum, plasma, or other body fluids. This kit is planed for high-throughput blood genomic DNA purification using 96 binding plates containing glass fiber-based membranes. The metaBIOn Blood Genomic DNA HTP Prep Kit is based on Proteinase K-lysis method; Lysis is achieved by incubation of whole blood under the solution containing Proteinase K at 56 °C. Appropriate conditions for binding of DNA to the membrane in the 96 binding plate are created by addition of Column Binding Solution. Ethanol-based Washing Solution removes contaminations. Pure genomic DNA is finally eluted under Tris buffer (10 mM Tris-HCl, pH 8.0. not provided) or deionized water. The obtained DNA can be used directly for PCR, southern blotting, or any kinds of enzymatic reactions.

B. Kit contents

Reagents for	**10 plates**	**30 plates**	3 plates (Testkit)
Nuclei Lysis Solution	120 ml	350 ml	35 ml
Column Binding Solution	240 ml	700 ml	70 ml
Column Washing Solution	480 ml	1400 ml	140 ml
Proteinase K (20mg/ml)	1.1ml x5 ea	1.1ml x16 ea	1.1ml x2 ea
96 well Binding filter	10 plates	30 plates	3 plates
96 well collection plates	10 plates	30 plates	3 plates
Sealing film	10 plates	30 plates	3 plates

C. Required Equipment

Vortex or plate shaker
Nuclease-free water (pH 7-8) or 10 mM Tris-HCl (pH 8.0)
Vacuum manifold or Plate centrifuge

D. Storage conditions

The metaBIOn Blood Genomic DNA HTP Prep Kit should be stored at room temperature without Proteinase K. Proteinase K solution is stable at 4 °C for up to 6 months. If the solution is not used up during this period, divide the solution into small aliquots and store at -20 °C before the first use of the kit.

E. Quality control

Manufacturing of all components is performed under clean conditions. Delivered kits are quality controlled by tests like restriction enzyme assay, spectrophotometric analysis and PCR analysis.

F. Detailed protocol

Precautions:

- **Wear gloves to avoid contact with all reagents.**
- **If eye or skin contact occurs, wash thoroughly with water**
- **Heat a incubator for use in <Step 5>.**
- **Check Nuclei Lysis Solution. If precipitated, heat to 55°-65°C for 5 minutes to dissolve.**
- **Perform all centrifuge steps at 3,000 rpm in microcentrifuge at room temperature.**

Version 1 [using a vacuum manifold]

1. Preparations from different samples for downstream steps.

Whole blood, body fluid	a. Only adjust the volume to 100 µl with PBS. b. Go to the <Step 2>
Cultured cells and lymphocytes	a. Harvest cells by centrifugation at 1500 x g for 10 minutes. b. Discard supernatant and resuspend cell pellet with 100 µl of PBS c. Go to the <Step 2>
Nucleated blood of fish, amphibian	a. Mix 10 µ of nucleated blood and 90 µl of PBS b. Go to the <Step 2>
Buccal swab	a. Gather the swab b. Add 100 µl of PBS to the scraped swap. c. Go to the <Step 2>

2. Pipet 5 µl of Proteinase K solution (20 mg/ml) into the bottom of each 96 well of plates.

3. Add 100 µl of sample to the wells. Use up to 100 µl of whole blood, plasma, buffy coat, serum or other body fluids.
For small volumes of sample, adjust the volume to 100 µl with PBS.

(Optional) If RNA free eluate is desired, add 3 µl of an RNAse A solution (10 mg/ml), vortex briefly and incubate for 15 min at 37 °C.

4. Add 100 µl Nuclei Lysis Solution to the sample, Mix immediately and vigorously (maximum speed) using a plate shaker or vortex for 1 min. The sample and Nuclei Lysis Solution should be mixed completely to ensure efficient lysis.

5. Incubate at 56 °C for 10 min.
Longer incubation times have no effect on yield or quality of the purified DNA. To ensure lysis of cells, vortex the plates every 2-3 min during incubation.

6. Place the binding plate on top of the manifold, and adjust the vacuum to 250 mmbar. Do not excess 250 mmbar vacuum setting during filtration of the lysate to ensure uniform filtration.

7. Carefully transfer the mixture to a binding plate, Apply the vacuum for 5-10 min. If the lysate is remained in binding plate, apply the vacuum, until all the mixture is completely passed through the membrane.

8. Add 200 µl of Binding Solution to each well of the Binding Plate. Apply full vacuum for 1 min.

9. Add 200 µl of Wash Buffer to each well of the Binding Plate. Apply full vacuum for 1 min.

10. Repeat <step 9>, but apply vacuum for 3 min.

11. Remove the Binding Plate from the manifold. Tap the plates firmly on several layers of paper towels on the bench to remove residual alcohol.

12. Apply the vacuum for 3 min to remove residual alcohol.

13. Place the Binding Plate on top of a collection plate. Apply 50 to 100 µl of deionized water or 10 mM Tris-HCl, pH 8.0 (not provided) directly in the middle of the Binding Plate membrane. Let stand for 1min. Maximum recovery is obtained with nuclease-free water warmed to 60-70 °C.

Version 2 [using a centrifuge]

1-5. Same protocol with step 1-5 in using vacuum.

6. Place the binding Plate on top of a deep well Plate.

7. Carefully transfer the mixture to a binding plate, centrifuge at 3,000 rpm for 5 min and change the deep well plate with new one
If the lysate is remained in the binding plate, centrifuge again at higher speed until all the mixture is completely passed through the membrane.

8. Add 200 µl of Column Binding Solution. Centrifuge at 3,000 rpm for 5 min. Discard the filtrated liquid and replace the binding filter on the deep well plate.

9. Add 200 µl Column Washing Solution and centrifuge at 3,000 rpm for 5 min. Discard the flow-through and replace the binding filter on the deep well plate.

10. Repeat the <Step 9> with 200 µl Column Washing Solution.

9. Place the Binding plate on top of a collection tube. Apply 50 to 100 µl of deionised water or 10 mM Tris-HCl, pH 8.0 (not provided) directly in the middle of the Binding plate membrane. Let stand for 1 min. Maximum recovery is obtained with nuclease-free water warmed to 60-70 °C.

10. Centrifuge at 2000x g for 5 min.

11. Centrifuge for an additional 5 min to remove residual Washing Solution.
If residual Washing Solution is not completely removed, subsequent enzymatic reactions may be inhibited.

12. Place the Binding Plate on top of a collection plate. Apply 50 to 100 µl of deionized water or 10 mM Tris-HCl, pH 8.0 (not provided) directly in the middle of the Binding Plate membrane. Let stand for 1min. Maximum recovery is obtained with nuclease-free water warmed to 60-70 °C.

13. Centrifuge at 3000 rpm for 5 min.

G. Hints and troubleshooting

Symptoms	Possible Causes	Comments
No or poor DNA yield	Low concentration of cells in sample	Major source of eluted DNA is white blood cells in whole blood. If concentration of white blood cells is low, DNA yield is also decreased. In this case, prepare buffy coat, leukocyte-enriched fraction of whole blood. For preparation of buffy coat, centrifuge whole blood at room temperature (2.500 x g, 10 min). Three different layers will be visible after centrifugation. Leukocytes are concentrated in the intermediate layer (= buffy coat)
	Incomplete cell lysis	Sample not thoroughly mixed with Nuclei Lysis Solution and Proteinase K. The mixture should be vortexed vigorously and immediately after addition of Nuclei Lysis Solution. Perform the procedure with freshly prepared Proteinase K. Store the Proteinase K at 4°C after use. Never add Proteinase K directly to Nuclei Lysis Solution.
	Unsuitable Elution buffer	Elution efficiency is dependent on pH and salt concentration. The optimal efficiency is obtained pH 7.0~8.5 and in the presence of low-salt buffer (ex. 10 mM Tris-HCl, pH 8.0 or D.W). To ensure yield, preheat elution buffer to 70 °C before elution. Apply Tris buffer or D.W directly onto the center of silica membrane.
Poor enzymatic reaction	Too high salt concentration in eluate	The common choice is repetition of wash step. Alternatively, leave the Spin Column for 5 min at room temperature after adding Washing Solution.
	Low purity of DNA	Inefficient cell lysis due to decreased protease activity. Perform the procedure with freshly

		prepared Proteinase K.
	Incompletely removal of hemoglobin	Inefficient cell lysis due to decreased protease activity. Perform the procedure with freshly prepared Proteinase K.
	RNA in eluate	RNA contamination is visible on gel and can be detected with high A260/280 ratio. RNA may inhibit subsequential enzymatic reactions. If RNA-free eluate is desired, add 3 µl of an RNAse A solution (10 mg/ml), vortex briefly and incubate for 15min at 37 °C before addition of Nuclei Lysis Solution.
	Residual Washing Solution in eluate	Centrifuge for an additional 5 min to remove residual Washing Solution.
Degraded DNA	Sample is too old or mis-stored.	Old and misstored samples lead to degrade DNA.