ATP Biotech Inc.

**ATP™ Reagent Genomic DNA Kit**
**Catalog No. AGE100**

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## Contents



# ATP™ Reagent Genomic DNA Kit

Store at room temperature (15~25℃)

1. ATPTM provide RBC Lysis Buffer to remove non-nucleated red blood cells and reduce hemoglobin contamination. But when blood sample is less than 50 μl or sample is nucleated red blood cells, we recommend using Cultured Cells protocol to purify genomic DNA.
2. ATP™ provide genomic DNA extraction protocols for smaller sample quantity. If larger sample quantity is required, user can scale up the buffer volume of the protocols proportionally.

## Blood Protocol (50～300 μl)

Additionally required：Microcentrifuge tube、Isopropanol、70 % Ethanol、RNase A (10 mg/ml)

### RBC Lysis (Use fresh blood)

1. Collected fresh blood in EDTA-NA2-treated collection tubes (or other anticoagulant).
2. Apply up to 300 μl of blood to a 1.5ml microcentrifuge tube. If blood sample is more than 300 μl blood (up to 1 ml), apply the blood sample to a sterile 15ml centrifuge tube.
3. Add 3X the sample volume of RBC Lysis Buffer and mix by inversion. Do not vortex.
4. Incubate the tube for 5 min at room temperature.
5. Centrifuge for 2 min at 3000 x g.
6. Remove the supernatant, but retain about 50 μl residual Buffer to resuspend the white cell pellet by vortexing.

### Cell Lysis

7. Add 300 μl Cell Lysis Buffer to the tube and mix by vortexing.
8. Incubate at 60 ℃ for 10 minutes until the sample lysate is clear. During incubation, invert the tube every 3 minutes.

Optional Step: RNA degradation (If RNA-free genomic DNA is required, perform this optional step.)
- Add 2 μl of RNase A (10 mg/ml, provided by user) to sample lysate and mix by vortexing.
- Incubate at room temperature for 10 minutes.

### Protein Remove

9. Add 100 μl of Protein Remove Buffer to the sample lysate and mix immediately by vortexing for 10 seconds.
10. Incubate on ice for 5 minutes.
11. Centrifuge at full speed (13,000 rpm) for 3 minutes.

### DNA Precipitation

12. Transfer the supernatant from Step 11 to a microcentrifuge tube.
13. Add 300 μl of Isopropanol and mix well by inverting.
14. Centrifuge at full speed (13,000 rpm) for 3 minutes.
15. Discard the supernatant and add 300 μl of 70% ethanol to wash the pellet.
16. Centrifuge at full speed (13,000 rpm) for 1 minutes.
17. Discard the supernatant and air-dry the pellet for 10 minutes.

### DNA Rehydration

18. Add 50-100 μl of Water or TE buffer and incubate at 60℃ for 30-60 minutes to dissolve DNA pellet. During incubation, tap the bottom of tube to promote DNA rehydration.

## Blood Protocol (300 μl～3ml)

Additionally required：15 ml centrifuge tube、Isopropanol、70 % Ethanol、RNase A (10 mg/ ml)

### RBC Lysis (Use fresh blood)

1. Collected fresh blood in EDTA-NA2-treated collection tubes (or other anticoagulant).
2. Apply up to blood sample (up to 3ml) to a sterile 15ml centrifuge tube.
3. Add 3X the sample volume of RBC Lysis Buffer and mix by inversion. Do not vortex.
4. Incubate the tube for 5 min at room temperature.
5. Centrifuge at 2000 x g for 5 min.
6. Remove the supernatant, but retain about 300 μl residual Buffer to resuspend the white cell pellet by vortexing.

### Cell Lysis

7. Add 3ml Cell Lysis Buffer to the tube and mix by vortexing.
8. Incubate at 60 ℃ for 10 minutes until the sample lysate is clear. During incubation, invert the tube every 3 minutes.

Optional Step: RNA degradation (If RNA-free genomic DNA is required, perform this optional step.)

- Add 10 μl of RNase A (10 mg/ml, provided by user) to sample lysate and mix by vortexing.
- Incubate at room temperature for 10 minutes.

### Protein Remove

9. Add 1ml of Protein Remove Buffer to the sample lysate and mix immediately by vortexing for 10 seconds.
10. Incubate on ice for 5 minutes.
11. Centrifuge at full speed (13,000 rpm) for 5 minutes.

### DNA Precipitation

12. Transfer supernatant (about 4 ml) from Step11 to a 15ml centrifuge tube.
13. Add 3ml of Isopropanol and mix well by inverting.
14. Centrifuge at full speed (13,000 rpm) for 5 minutes.
15. Carefully remove the supernatant and add 3ml of 70% ethanol to wash the pellet.
16. Centrifuge at full speed (13,000 rpm) for 1 min.
17. Discard the supernatant and air-dry the pellet for 20 minutes.

### DNA Rehydration

18. Add 100-300 μl of Water or TE buffer and incubate at 60℃ for 30-60 minutes to dissolve DNA pellet. During incubation, tap the bottom of tube to promote DNA rehydration.



## Blood Protocol (3ml～10ml)

Additionally required：50 ml centrifuge tube、Isopropanol、70 % Ethanol、RNase A (10 mg/ ml)

### RBC Lysis (Use fresh blood)

1. Collected fresh blood in EDTA-NA2-treated collection tubes (or other anticoagulant).
2. Apply up to blood sample (up to 10 ml) to a sterile 50ml centrifuge tube.
3. Add 3X the sample volume of RBC Lysis Buffer and mix by inversion. Do not vortex.
4. Incubate the tube for 5 min at room temperature.
5. Centrifuge at 2000 μg for 5 min.
6. Remove the supernatant, but retain about 500 μl residual Buffer to resuspend the white cell pellet by vortexing.

### Cell Lysis

7. Add 10ml Cell Lysis Buffer to the tube and mix by vortexing.
8. Incubate at 60 ℃ for 10 minutes until the sample lysate is clear. During incubation, invert the tube every 3 minutes.

Optional Step: RNA degradation (If RNA-free genomic DNA is required, perform this optional step.)

- Add 20 μl of RNase A (10 mg/ml, provided by user) to sample lysate and mix by vortexing.
- Incubate at room temperature for 10 minutes.

### Protein Remove

9. Add 3.3 ml of Protein Remove Buffer to the sample lysate and mix immediately by vortexing for 10 seconds.
10. Incubate on ice for 5 minutes.
11. Centrifuge at full speed (13,000 rpm) for 3 minutes.

### DNA Precipitation

12. Transfer supernatant (about 4 ml) from Step 11 to a 15 ml centrifuge tube.
13. Add 10 ml of Isopropanol and mix well by inverting.
14. Centrifuge at full speed (13,000 rpm) for 5 minutes.
15. Carefully remove the supernatant and add 10ml of 70% ethanol to wash the pellet.
16. Centrifuge at full speed (13,000 rpm) for 1 min.
17. Discard the supernatant and air-dry the pellet for 20 minutes.

### DNA Rehydration

18. Add 300-600 μl of Water or TE buffer and incubate at 60 ℃ for 30-60 minutes to dissolve DNA pellet. During incubation, tap the bottom of tube to promote DNA rehydration.

## Culture Cells Protocol

Additionally required：Microcentrifuge tube、Isopropanol、70 % Ethanol、RNase A (10 mg/ ml)

### Sample Preparation

**(Cultured animal cells)**

If use adherent cells, trypsinize the cells before harvesting.

A. Transfer $10^6$-$10^7$ of cells to a microcentrifuge tube (not provided) and harvest the cells with centrifugation for 20 seconds at 6,000 x g (about 8,000 rpm for microcentrifuge).

B. Remove the supernatant, but retain about 50 μl residual buffer to resuspend the white cell pellet by vortexing.

**(Blood)**

For mammalian blood (non-nucleated), the sample volume is up to 50 μl.

For nucleated erythrocytes (e.g., bird or fish), the sample volume is up to 10 μl.

A. Collected fresh blood in EDTA-NA2-treated collection tubes (or other anticoagulant).

### Cell Lysis

1. Add 300 μl Cell Lysis Buffer to the sample and mix by vortexing.
2. Incubate at 60 ℃ for 10 minutes until the sample lysate is clear. During incubation, invert the tube every 3 minutes.

Optional Step: RNA degradation (If RNA-free genomic DNA is required, perform this optional step.)

- Add 4 μl of RNase A (10 mg/ml, provided by user) to sample lysate and mix by vortexing.
- Incubate at room temperature for 5 minutes.

### Protein Remove

3. Add 100 μl of Protein Remove Buffer to the sample lysate and mix immediately by vortexing for 10 seconds.
4. Incubate on ice for 5 minutes.
5. Centrifuge at full speed (13,000 rpm) for 3 minutes.

### DNA Precipitation

6. Transfer the supernatant from Step5 to a microcentrifuge tube.
7. Add 300 μl of Isopropanol and mix well by inverting.
8. Centrifuge at full speed (13,000 rpm) for 3 minutes.
9. Discard the supernatant and add 300 μl of 70% ethanol to wash the pellet.
10. Centrifuge at full speed (13,000 rpm) for 3 minutes.
11. Discard the supernatant and air-dry the pellet for 10 minutes.

### DNA Rehydration

12. Add 50-100 μl of Water or TE buffer and incubate at 60 ℃ for 30-60 minutes to dissolve DNA pellet. During incubation, tap the bottom of tube to promote DNA rehydration.



## Gram-positive Bacterial Protocol

If sample is gram-negative bacteria, use Cultured Cells Protocol.

Additionally required：Lysozyme Buffer (20 mg/ml lysoyme；20 mM Tris-HCl；2 mM EDTA；1% Triton X-100，pH 8.0)，Prepare the lysozyme buffer just before use

### Cell Harvest / Prelysis (For Gram-positive bacteria)

1. Transfer bacterial culture (< $10^9$) to a microcentrifuge tube (not provided).
2. Centrifuge for 1 min at full speed (13,000 rpm) in a microcentrifuge and discard the supernatant.
3. Add 100 μl of Lysozyme Buffer to the tube and resuspend the cell pellet by vortexing or pipetting.
4. Incubate at room temperature for 20 minutes. During incubation, invert the tube every 2-3min.
5. Proceed with Step1 Cell Lysis of Cultured Cells protocol.

## Yeast Protocol

Additionally required：Sorbitol buffer (1.2M sorbitol ； 10mM $CaCl_2$ ； 0.1M Tris/Cl，pH 7.5；35mM mercaptoethanol)

Lyticase or zymolase

### Cell Harvest / Prelysis (For Gram-positive bacteria)

1. Harvest yeast cells (up to 5 x $10^7$) by centrifugation for 10 min at 5,000g.
2. Resuspend the pellet in 600 μl sorbitol buffer.
3. Add 200U of lyticase or zymolase. Incubate at 30 ℃ for 30 min.
4. Centrifuge the mixture for 10 min at 2,000g to harvest Spheroplast.
5. Proceed with Step1 Cell Lysis of Cultured Cells protocol.


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