# Axen™ Total DNA BYC mini Kit

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## Product description

Axen™ Total DNA BYC mini kit is designed for simple and rapid purification of total DNA from various sample sources including animal tissues and cells, blood, bacteria, or yeast. Purified DNA is free of contaminants and enzyme inhibitors and is highly suited for downstream applications such as PCR, blotting, and enzymatic reactions.

## Kit contents

| Contents* | | | Storage conditions |
|---|---|---|---|
| Cat. No. | **MG-P-006-50** | **MG-P-006-200** | |
| No. of preparation | 50 | 200 | |
| Mini column and collecting tubes | 50 ea | 200 ea | Room Temperature |
| Buffer TL | 15 mL | 45 mL | |
| Buffer BL | 15 mL | 45 mL | |
| Buffer AW (conc.)** | 18 mL | 70 mL | |
| Buffer TW (conc.)** | 8 mL | 30 mL | |
| Buffer EA*** | 15 mL | 50 mL | |
| Buffer GPL | 15 mL | 50 mL | |
| Buffer YCL | 30 mL | 110 mL | 4˚C |
| Proteinase K solution (20 mg/mL)**** | 1.05 mL | 4.2 mL | |

* All components of this kit except Proteinase K solution should be stored at room temperature (15~25°C). Long exposure to heat source can deteriorate the performance of kit significantly.

** Wash buffers are provided as concentrates. Ethanol must be added before first use as the indication on the bottle labels.

*** 10 mM Tris-HCl, pH 9.0, 0.5 mM EDTA

**** Proteinase K should be stored under 4°C on arrival for conservation of activity. Delivery time would not lead to noticeable loss of the enzyme's activity. Proteinase K solution can be stored at 4°C for a year without significant decrease of performance.

## Product use limitations

Axen™ Total DNA BYC mini kit is intended for research uses only. This kit is not intended for diagnosis or treatment for human. All due care and attention should be exercised in the handling of the products.

## Safety information

Axen™ Total DNA BYC mini Kit contains irritants which are harmful when in contact with skin or eyes, or inhaled or swallowed. Care should be taken when handling this product. Always wear gloves and eye protection, and follow standard safety precautions.

Buffer BL and buffer AW contain chaotropes which can form highly reactive compounds when combined with bleach. Do NOT add bleach or acidic solutions directly to the sample-preparation waste.

## Preparation of ethanol-added buffer

Buffer AW and TW are provided as concentrate. Absolute ethanol (ACS grade or better) should be added as below before first use.

| Buffer name | Included in | Volume of contents | **Ethanol to be added** | The final volume |
|---|---|---|---|---|
| AW | MG-P-006-50 | 18 mL | **18 mL** | 36 mL |
| | MG-P-006-200 | 70 mL | **70 mL** | 140 mL |
| TW | MG-P-006-50 | 8 mL | **32 mL** | 40 mL |
| | MG-P-006-200 | 30 mL | **120 mL** | 150 mL |

## Protocol for preparation of genomic DNA

***To check before start***

- Prepare water bath or heat block at 56°C
- Required materials; 1.5 mL centrifuge tube,
  Absolute ethanol,
  100 mg/mL RNase A(*optional*)
  Lysozyme or lyticase (*optional*)

**!!! Before first use, add the indicated volume of absolute ethanol to buffer AW and TW. (Refer to the *previous page*)**

## ● *Sample Preparation Procedure*

### A. Animal Cultured Cells

1. **Harvest up to 5 x $10^6$ cultured cells in a 1.5 mL tube by centrifugation.**
2. **Discard the supernatant as much as possible.**
3. **Apply 200 uL of buffer TL and resuspend the cell pellet completely.**
   - *For efficient resuspending in buffer TL, it is helpful to loosen and disrupt the pellets by flickering or vortexing before the addition of buffer TL.*
4. **Pipet 20 uL of Proteinase K solution (20 mg/mL) into the tube and mix completely by vortexing or pipetting.**
5. **Go to 'DNA Purification procedure'**

## B. Animal Tissues

### 1. Place up to 20 mg of a ground or minced tissue into a 1.5 mL tube.

- *Grind the tissue to a fine powder with liquid nitrogen in a pre-chilled mortar and pestle, or the tissue sample can be applied as minced with sterile sharp blade or scalpel as small as possible.*
- *Alternatively, a soft tissue can be homogenized in buffer TL using a micro-homogenizer.*
- *Do not exceed 10 mg for spleen or kidney.*

### 2. Apply 200 uL of buffer TL and 20 uL of Proteinase K solution (20 mg/mL) into the tube.

### 3. Mix completely by vortexing or pipetting.

- *It is essential for good result to mix the components thoroughly to make a homogenate.*

### 4. Incubate the tube at 56˚C until the tissue is completely lysed.

- *Complete lysis time varies from 10 mins to 3 hrs depending on the type of tissue. The lysate usually becomes translucent without any particulates after complete lysis. Overnight lysis does not influence the preparation.*
- *For efficient lysis, do vortex occasionally during incubation. Use of a specialized instrument such as themo-mixer will accelerate the lysis.*
- *Make sure the tissue is completely digested.*

### 5. Go to 'DNA Purification procedure'

## C. Whole Blood (Mammalian)

1. **Place up to 200 uL of whole blood sample into a 1.5 mL tube.**
   - *If a less sample than 200 uL is used, adjust the sample volume to 200uL with buffer TL.*
2. **Pipet 20 uL of Proteinase K solution (20 mg/mL) into the tube and mix completely by vortexing or pipetting.**
3. **Go to 'DNA Purification procedure'**

## D. Nucleated Blood

1. **Place up to 10 uL of nucleated blood sample with 190 uL of buffer TL into a 1.5 mL tube.**
2. **Pipet 20 uL of Proteinase K solution (20 mg/mL) into the tube and mix completely by vortexing or pipetting.**
3. **Go to 'DNA Purification procedure'**

## E. Gram Negative Bacteria

1. **Harvest up to 2 x $10^9$ bacterial cells in a 1.5 mL tube by centrifugation.**
2. **Discard the supernatant as much as possible.**
3. **Apply 200 uL of buffer TL and resuspend the pellet completely.**
   - *For efficient resuspending in buffer TL, it is helpful to loosen and disrupt the pellets by flickering or vortexing before the addition of buffer TL.*
4. **Pipet 20 uL of Proteinase K solution (20 mg/mL) into the tube and mix completely by vortexing or pipetting.**
5. **Go to 'DNA Purification procedure'**

## F. Gram Positive Bacteria

- ***Prepare water bath or dry bath of 37°C, 70°C and/or 95°C as needed***

1. **Prepare the enzyme mixture; Resuspend the lytic enzyme (not provided) in buffer GPL as listed below.**
   - *Enzyme mixture should be stored in small aliquots below -20°C; Ideally, once per an aliquot. Thawed aliquot should be discarded after use.*

| Enzymes | References | Working conc. | Instruction |
|---|---|---|---|
| Lysozyme | L6876, Sigma or equivalents | 30 mg/mL | Resuspend 30 mg of lysozyme in 1 mL of buffer GPL |
| Lysostaphin | L4402, Sigma or equivalents | 300 ug/mL | Resuspend 0.3 mg of lysostaphin in 1mL of buffer GPL |

   - *For Staphylococcus species, lysostaphin can be more effective than lysozyme. However, lysozyme is sufficient to lyse the cell walls for most gram-positive bacteria.*
2. **Harvest 2 x $10^9$ bacterial cells in a 1.5 mL tube by centrifugation. Discard the supernatant as much as possible.**
3. **Resuspend the pellet thoroughly in 180 uL of the prepared enzyme mixture from step 1.**
4. **Incubate for 30 mins at 37°C.**
5. **Add 20 uL of Proteinase K solution (20 mg/mL) and 200 uL of buffer BL into the tube and mix completely by vortexing or pipetting.**
6. **Incubate for 30 mins at 56°C and then for a further 30 mins at 70°C.**
   - *When a pathogen is subjected, it is strongly recommended to incubate for 15 mins at 90°C rather than for 30 mins at 70°C*
7. **Cool to room temperature and go to step 4 of 'DNA Purification Procedure'.**

## G. Yeast cells

1. **Harvest up to 5 x $10^7$ cells or 3 mL of a culture grown in YPD broth into a 15 mL conical tube by centrifugation at 10,000 xg for 5 mins. Discard carefully the supernatant as much as possible without disturbing the pellet.**
2. **Resuspend the pellet thoroughly in 500 uL of buffer YCL.**
3. **Add 200 U of lyticase (not provided) or 20 U of zymolase (not provided) and gently pipet to mix completely.**
   - *U/mL of enzyme varies depending on its manufacture.*
4. **Incubate at 37°C for 1 hour to digest the cell wall.**
5. **Centrifuge at 10,000 xg for 3 mins and discard carefully the supernatant as much as possible without disturbing the pellet.**
6. **Resuspend thoroughly the cell pellet in 200 uL of buffer TL.**
7. **Pipet 20 uL of Proteinase K solution into the tube, vortex vigorously to mix completely, and incubate 56°C for 15 mins.**
8. **Cool to room temperature and go to 'DNA Purification Procedure'.**

- ***DNA Purification Procedure***

1. **(*Optional*) If RNA should be removed, add 4 uL of RNase A solution (100 mg/mL, not provided) to the sample tube, vortex to mix and incubate for 2 mins at room temperature.**
2. **Add 200 uL of buffer BL and vortex to mix thoroughly.**
   - *It is essential for good result to mix the components thoroughly to make a homogenate.*
3. **Incubate the tube at 56˚C for 10 mins.**
4. **Apply 200 uL of absolute ethanol and briefly vortex to mix.**
5. **Transfer all of the mixture into a spin column, centrifuge at 10,000 xg for 1 min, empty the collecting tube and re-insert the spin column back to the tube.**
6. **Apply 600 uL of buffer AW to the spin column, centrifuge at 10,000 xg for 30 secs, empty the collecting tube and re-insert the spin column back to the tube.**
7. **Apply 600 uL of buffer TW to the spin column, centrifuge at 10,000 xg for 30 secs, empty the collecting tube and re-insert the spin column back to the tube.**
8. **Centrifuge the tube for 1 min at full speed for drying the membrane and transfer the spin column into a new 1.5 mL tube.**
   - *Residual ethanol in eluate may interfere with the subsequent reactions. If carryover of wash buffer occurs, centrifuge again for 1 min before proceeding to next step.*
9. **Apply 50 ~ 200 uL of buffer EA to the center of spin column membrane and incubate for 1 min at room temperature.**
   - *Make sure that the buffer EA is dispensed directly onto the center of column membrane.*
   - *Elution volume can be decreased to 50 ul for higher concentration of the eluted DNA, but this will slightly decrease in overall DNA yield. If maximum recovery of DNA is preferred or the starting materials contain large amount of DNA, the elution volume can be increased to 200 ul.*
10. **Centrifuge at full speed (>13,000 xg) for 1 min to elute the DNA.**

## Trouble shooting guide

| Facts | *Possible causes* | Recommendations |
|---|---|---|
| Low or no recovery | *Low cells in the starting sample* | Some samples may have low population of cells, and some whole blood may contain low numbers of white blood cells. Increase the sample volumes and load the binding mixture into the spin column several times. Reduce the elution volume to minimum. When the cell mass is very low, it is recommended to use a carrier RNA, which can be used by dissolving at 5~10 ug/mL in buffer BL. |
| | *Too many cells in the starting sample* | The sample amount over the maximum capacity will lead to poor lysis of cells, resulting in significantly low recovery. Reduce the amount of starting sample or increase the volume of buffers proportionally. |
| | *Inefficient lysis* | Some hard tissues such as heart and muscle take a long time to be completely lysed, and well shredded tissue will shorten this time. At start, pulverize the tissue to get homogenate. Using the thermo-mixer can greatly accelerate the lysis. Completely lysed mixture will not have any particulate on it. Degenerated Proteinase K may also cause inefficient lysis. Proteinase K should be stored under 4°C for maintenance of proper activity. |
| | *Improper eluent* | An eluent other than buffer EA can be employed as user's needs. The optimal eluting condition should be under low salt concentration with alkaline pH (7.0<pH<9.0). When water or other buffer was used as eluent, ensure that condition. |
| | *Ethanol was not added to the buffer* | Buffers AW and TW are provided as concentrate. Ethanol must be added to these buffers before first use. Otherwise, the result will be significantly poor. |

| | | |
|---|---|---|
| Low purity or the colored residue on spin column membrane | *Inefficient lysis* | Inefficient lysis of sample may result in low DNA purity, and it may be due to the reasons described upper section, '*Inefficient lysis*'. |
| | *Incomplete removal of hemoglobin* | In case of DNA preparation from a certain animal blood or from an improperly stored blood, it is hard to remove hemoglobin from their blood. Carry out additional wash step with buffer AW before washing with buffer TW. |
| High $A_{260}/A_{280}$ ratio | *RNA contamination* | RNA can inhibit some downstream enzymatic reactions, but not PCR itself. If RNA-free DNA is required, carry out RNase A treatment on step 1 of 'DNA Purification Procedure'. |
| Clogging of spin column | *Inefficient lysis* | Small particulates in incomplete lysate can clog the pore of the filter membrane. Please refer to the recommendation for inefficient lysis above. |
| Degraded DNA | *Starting sample is too old or improperly stored* | Too old or improperly stored samples have degraded DNA. Use fresh sample. |
| Enzymatic reaction is not performed well with purified DNA | *High salt concentration in eluate* | Ensure that wash step is carried out just in accordance with the protocols. Additional wash step may help to remove high salt in eluate. |
| | *Low purity of DNA eluate* | Please refer to the recommendation for 'low purity' above. |
| | *Residual ethanol in eluate* | Ensure that the wash step on the protocol is performed properly. The spin column membrane should be dried completely before eluting DNA. Perform an additional centrifugation to dry the membrane, if needed. |


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