# Axen™ GMO DNA mini Kit

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## Product description

Axen™ GMO DNA mini Kit provides the simple and rapid method for extraction of total DNA from various plant seeds or grains. Purified DNA is free of contaminants and enzyme inhibitors and is highly suited for downstream applications such as PCR, genotyping and various enzymatic reactions.

## Kit contents

| Contents* | | | Storage conditions |
|---|---|---|---|
| Cat. No. | **MG-P-009-50** | **MG-P-009-200** | |
| No. of preparation | 50 | 200 | |
| Mini column and collecting tubes | 50 ea | 200 ea | Room Temperature |
| Buffer GML | 55 mL | 220 mL | |
| Buffer GMB | 30 mL | 110 mL | |
| Buffer AW (conc.)** | 20 mL | 75 mL | |
| Buffer TW (conc.)** | 16 mL | 30 mL x 2 | |
| Buffer EA*** | 15 mL | 50 mL | |
| Proteinase K solution (20 mg/mL)**** | 0.55 mL | 1.05 mL x 2 | 2 ~ 8˚C |

* 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.

** Buffer AW and TW are provided as concentrates. Absolute ethanol (ACS grade or better) must be added before first use as indicated 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™ GMO DNA 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™ GMO DNA 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 GMB 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-009-50 | 20 mL | **20 mL** | 40 mL |
| | MG-P-009-200 | 75 mL | **75 mL** | 150 mL |
| **TW** | MG-P-009-50 | 16 mL | **64 mL** | 80 mL |
| | MG-P-009-200 | 30 mL x 2 | **120 mL x 2** | 150 mL x 2 |

## Procedure for preparation of DNA

***To check before start***

- Prepare water bath or heat block at 65°C
- Required materials; 1.5 mL or 2.0 mL centrifuge tube

☞ All centrifugation steps should be performed at full speed (>10,000 xg, 10,000 ~ 14,000 rpm) in a microcentrifuge at room temperature.

☞ Do not use a precipitated buffer. If a precipitate has been formed in any buffer, dissolve completely at 20°C ~ 40°C before use.

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

1. **Place up to 200 mg of a ground sample into a 2.0 mL microcentrifuge tube.**
   - *Grind fresh or frozen plant tissue to a fine powder quickly and completely, using a mortar and pestle pre-cooled with liquid nitrogen. Quick and complete pulverization at low temperature is essential for good result.*
   - *Mortar and pestle is a good conventional method for pulverizing, but other methods like a bead-beating instrument or a rotor-stator homogenizer can be good alternatives. Follow the instruction manuals for those methods.*
2. **Add 1 mL of buffer GML and 10 uL of Proteinase K solution (20 mg/ml) into the tube and vortex vigorously for 15 secs.**
   - *Mix completely to make the lysate homogenate without any clumps.*
3. **Incubate for 30 mins at 65℃.**
   - *Occasional vortex during incubation may accelerate the lysis. Use of a specialized instrument such as thermo-mixer will accelerate the lysis.*
4. **Centrifuge for 5 mins and transfer 500 uL of supernatant into a new 1.5 mL microcentrifuge tube.**
   - *Be careful not to co-transfer the debris as much as possible.*

5. **Apply an equal volume (500 uL) of buffer GMB into the tube and vortex for 10 secs to mix.**
6. **Centrifuge for 5 mins.**
7. **Transfer 700 uL of the cleared supernatant into a spin column, centrifuge for 30 secs, discard the passed-through, and re-insert the column back into the tube.**
   - *Be careful not to co-transfer the debris. It can cause clogging of spin column.*
8. **Repeat step 7 with the remaining sample.**
9. **Apply 650 ul of buffer AW to the spin column, centrifuge for 30 secs, empty the collecting tube and re-insert the spin column back to the tube.**
10. **Apply 650 ul of buffer TW to the spin column, centrifuge for 30 secs, empty the collecting tube and re-insert the spin column back to the tube.**
11. **Repeat step 10 once.**
12. **Centrifuge the tube for 2 mins for drying the membrane and transfer the spin column into a new 1.5 mL microcentrifuge 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.*
13. **Apply 50 ~ 100 ul of buffer EA onto the center of spin column membrane and incubate for 5 mins at room temperature. Centrifuge for 1 min for eluting the DNA.**
    - *Elution volume can be decreased to 30 ul for higher concentration of 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.*

## Trouble shooting guide

| Facts | *Possible causes* | Recommendations |
|---|---|---|
| Low recovery | *Too much starting materials* | Too much starting materials may bring about inefficient lysis and spin column clogging, followed by poor DNA yields. Reduce the amount of starting material as described on procedure. |
| | *Too old or misplaced sample used* | DNA can be degraded, especially when the tissues are too old or incorrectly stored. Use fresh samples. |
| | *Insufficient disruption/lysis* | Pulverizing of the sample is critical step for good result. Improperly disrupted sample will result in poor lysis, followed by poor yield. Pulverize completely the tissue to get homogenate. |
| | *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. If not, the result will be significantly poor. |
| | *Improper elution* | As user's need, elution buffer other than buffer EA can be engaged. However, the conditions of optimal elution should be low salt concentration with weak alkaline pH (7<pH<9). When water or other buffer was used as eluent, ensure that condition. After eluent is applied on the center of spin column membrane, it is essential to incubate at least for 5 minutes at room temperature. |
| Low purity | *Contamination of debris* | Any cell debris or precipitates must be excluded when transferring the supernatant at step 4 and step 7 |
| | *Insufficient lysis* | Too much starting material can lead to poor lysis, followed by low purity of DNA. |
| Clogging of spin column | *Incomplete removal of precipitate* | Any cell debris or precipitates must be excluded when transferring the supernatant at step 4 and step 7, because these can clog the pores of spin column membrane. |
| | *Lysate too viscous or sticky* | Reduce the amount of starting sample, or increase the amount of buffers proportionally. |
| Degraded DNA | *Too old or misplaced sample used* | DNA can be degraded, especially when the tissues are too old or incorrectly stored. Use fresh samples. |
| | *Excessive or retarded shredding* | It is essential for good result to pulverize the sample thoroughly. However, excessive or retarded shredding of sample will lead to damage on DNA. |
| 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 TW-washing can help remove salts from the membrane. |
| | *Residual ethanol in eluate* | The spin column membrane should be dried completely before eluting DNA. Perform additional centrifugation to dry the membrane, if needed. |


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