Axen™ DNA Micro Kit

Product description

Axen™ DNA Micro Kit is designed for simple and rapid purification of total DNA from various samples sources which have very low contents of nucleic acid, including a research, clinical or forensic sample. Purified DNA is free of contaminants and enzyme inhibitors and is highly suited for downstream applications such as PCR, qPCR, genotyping such as STR analysis, and other enzymatic reactions.

Kit contents

Contents [a]			Storage conditions
Cat. No.	**MG-P-007-50**	**MG-P-007-200**	
No. of preparation	50	200	
Micro spin columns [b]	50 ea	200 ea	**2~8°C** [b]
Buffer AL	20 mL	75 mL	RT
Buffer BL	20 mL	75 mL	RT
Buffer AW (conc.) [c]	18 mL	60 mL	RT
Buffer TW (conc.) [c]	10 mL	36 mL	RT
Buffer EA [d]	15 mL	50 mL	RT
Carrier RNA [e]	300 ug	1.1 mg	**2~8°C** [b]
Proteinase K solution (20 mg/ml) [f]	1.05 mL	4.2 mL	**2~8°C** [b]

a. Check the storage conditions for each item upon arrival. Long exposure to heat source can deteriorate the performance of kit significantly.

b. Avoid heat source and direct sunlight.

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

d. 10 mM Tris-HCl, pH 9.0, 0.5 mM EDTA

e. Carrier RNA should be stored under 4˚C upon arrival for avoidance of degradation. After the reconstitution of carrier RNA solution, it is ideal to store under -20˚C with dividing into small aliquot. Repeated freezing and thawing will degrade the RNA.

f. 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 an year without significant decrease of performance, but it can be stored under -20˚C for prolonged conservation of activity.

Product use limitations

Axen™ DNA micro 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™ DNA micro 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.

Reconstitution of carrier RNA solution

Carrier RNA is provided in a lyophilized form. In order to reconstitute the carrier RNA solution to 1 ug/uL, add 1 uL of distilled water per 1 ug of carrier RNA. For instance, add 300 uL of distilled water into 300 ug of carrier RNA.

The reconstituted carrier RNA solution should be stored at low temperature for avoidance of degradation. It is ideal to store under -20°C with dividing into small aliquot. Note that repeated freezing and thawing will degrade the RNA.

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-007-50	18 ml	**18 ml**	36 ml
	MG-P-007-200	60 ml	**60 ml**	120 ml
TW	MG-P-007-50	10 ml	**40 ml**	50 ml
	MG-P-007-200	36 ml	**144 ml**	180 ml

Protocol for DNA preparation

To check before start

- Prepare water bath or heat block at 56°C and/or 70°C
- Required materials; 1.5 ml centrifuge tube, Absolute ethanol, 1M DTT (See the protocol before proceeding)
- All centrifugation steps should be performed at full speed (>10,000 xg, 10,000 ~ 14,000 rpm) in a microcentrifuge at room temperature.

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

● Sample Preparation Procedure

- *If very low yield is expected, use of carrier RNA is strongly recommended.*
- *Add 5 uL of carrier RNA solution(0.2 ug/uL) per prep into a lysate mixture or buffer BL.*

A. Small volume of mammalian whole blood

1. **Pipet 10 uL of Proteinase K solution into the bottom of a 1.5 mL tube.**
2. **Transfer 1 ~ 100 uL of whole blood to the tube**
   - *When the volume of blood is less than 100 uL, adjust the volume to 100 uL with buffer AL.*
3. **Add 100 uL of buffer BL to the tube and vortex for 15 secs to mix thoroughly.**
   - *When the sample is less than 10 uL of whole blood, it is recommended to add carrier RNA to buffer BL. Use 5 uL of carrier RNA solution (1ug/uL) per prep.*

4. **Incubate the tube at 56˚C for 10 mins.**
5. **Add 100 uL of absolute ethanol to the tube, vortex twice for 5 secs to mix.**
6. **Go to 'DNA Purification procedure'**

B. Swab (buccal, vaginal or skin)

1. **Place the head of swab into a 1.5 mL tube.**
2. **Add 300 uL of buffer AL and 20 uL of proteinase K and vortex the tube for 10 secs to mix.**
   - *When the sample is a semen swab, apply 20 uL of 1M DTT to the tube additionally.*
3. **Incubate the tube at 56˚C for 1 hour.**
   - *For efficient lysis, vortex the tube every 10 mins during the incubation. Use of a specialized instrument such as thermo-mixer will accelerate the lysis.*
4. **Add 300 uL of buffer BL to the tube and vortex for 15 secs to mix thoroughly.**
5. **Incubate the tube at 70˚C for 10 mins.**
6. **Add 300 uL of absolute ethanol to the tube, vortex twice for 5 secs to mix.**
7. **Go to 'DNA Purification procedure'**

C. Body Fluid Stains (blood, saliva or semen)

1. **Place 0.5 cm^2 of punched-out disc from stained materials into a 1.5 mL tube.**
2. **Add 200 uL of buffer AL and 20 uL of proteinase K and vortex the tube for 10 secs to mix.**
   - *When the sample is semen swab, apply 20 uL of 1M DTT to the tube additionally.*

3. **Incubate the tube at 56˚C for 1 hour.**
   - *For efficient lysis, vortex the tube every 10 mins during the incubation. Use of a specialized instrument such as thermo-mixer will accelerate the lysis.*
4. **Add 200 uL of buffer BL to the tube and vortex for 15 secs to mix thoroughly.**
5. **Incubate the tube at 70˚C for 10 mins.**
6. **Add 200 uL of absolute ethanol to the tube, vortex twice for 5 secs to mix.**
7. **Go to 'DNA Purification procedure'**

D. Hair and Nail Clippings

1. **Place hairs(root, shaft) or nail clippings into a 1.5 mL tube.**
   - *The shaft of hairs does not contain any genomic DNA. It has only mitochondrial DNA. Use the hair roots for the recovery of genomic DNA.*
2. **Add 200 uL of buffer AL, 20 uL of proteinase K, and 20 uL of 1M DTT, and vortex the tube for 15 secs to mix thoroughly.**
3. **Incubate the tube at 56˚C for 1 hour or until the sample is dissolved completely.**
   - *For efficient lysis, vortex the tube every 10 mins during the incubation. Use of a specialized instrument such as thermo-mixer will accelerate the lysis.*
   - *Especially for complete lysis of nail clippings, overnight incubation is recommended.*
4. **Add 200 uL of buffer BL to the tube and vortex for 15 secs to mix thoroughly.**
5. **Incubate the tube at 70˚C for 10 mins.**
6. **Add 200 uL of absolute ethanol to the tube, vortex twice for 5 secs to mix.**
7. **Go to 'DNA Purification procedure'.**

E. Bones and Teeth

1. **Disrupt bones or teeth sample using one of the following methods.**

   - *Using SPEX Freezer Mill*

     Transfer the small fragment of bones or teeth and the ball into a grinding vial. Put the vial into Freezer Mill then pour liquid nitrogen. Grind the bone or teeth until the sample is pulverized completely. Refer to the instruction manual of the instrument for the detail.

   - *Using the metal blender*

     Crush the bones or teeth into small fragment. Grind to a fine powder using a metal blender half-filled with liquid nitrogen. Refer to the instruction manual of the instrument for the detail.

   - *Using EDTA*

     Transfer the bones or teeth into centrifuge tube. Pour 0.5 M EDTA solution to sink the sample. Incubate the sample for decalcification at room temperature until the sample become flexible (for several days or even weeks, depending on the size of the bones or teeth). Change the EDTA solution occasionally during incubation. Cut the sample as small as possible with a sterile microtome or blade.

2. **Place up to 100 mg of bones or teeth into a 1.5 mL tube.**
3. **Add 300 uL of buffer AL and 20 uL of proteinase K, and vortex the tube for 15 secs to mix thoroughly.**
4. **Incubate the tube at 56˚C overnight.**
   - *For efficient lysis, shaking or vortexing bath is recommended. Use of a specialized instrument such as thermo-mixer will accelerate the lysis.*
5. **Add 300 uL of buffer BL to the tube and vortex for 15 secs to mix thoroughly.**
6. **Incubate the tube at 70˚C for 10 mins.**
7. **Centrifuge for 1 min, and carefully transfer the supernatant to a new 1.5 mL tube.**
8. **Add 300 uL of absolute ethanol to the tube, vortex twice for 5 secs to mix.**
9. **Go to 'DNA Purification procedure'**

F. Cigarette Butts

1. **Cut out a 1 cm^2 piece of outer paper from the end of the cigarette or filter. Cut this piece into 6 smaller piecies. Transfer the piecies to a 1.5 mL tube.**
2. **Add 300 uL of buffer AL and 20 uL of proteinase K and vortex the tube for 10 secs to mix.**
3. **Incubate the tube at 56˚C for 1 hour.**
   - *For efficient lysis, vortex the tube every 10 mins during the incubation. Use of a specialized instrument such as thermo-mixer will accelerate the lysis.*
4. **Add 300 uL of buffer BL to the tube and vortex for 15 secs to mix thoroughly.**
5. **Incubate the tube at 70˚C for 10 mins.**
6. **Add 300 uL of absolute ethanol to the tube, vortex twice for 5 secs to mix.**
7. **Go to 'DNA Purification procedure'**

G. Tooth Brush

1. **Collect bristles on tooth brush in a 1.5 mL tube.**
   - *Alternatively, rinse the tooth brush with 10 mL of 1x PBS and collect the buccal cells by centrifugation.*
2. **Add 200 uL of buffer AL and 20 uL of proteinase K, and vortex the tube for 15 secs to mix thoroughly.**
3. **Incubate the tube at 56˚C for 1 hour.**
   - *For efficient lysis, vortex the tube every 10 mins during the incubation. Use of a specialized instrument such as thermo-mixer will accelerate the lysis.*
4. **Add 200 uL of buffer BL to the tube and vortex for 15 secs to mix thoroughly.**
5. **Incubate the tube at 70˚C for 10 mins.**

6. **Add 200 uL of absolute ethanol to the tube, vortex twice for 5 secs to mix.**
7. **Go to 'DNA Purification procedure'.**

H. Animal Tissue

1. **Transfer up to 10 mg of an animal tissue to a 1.5 mL tube.**
2. **Add 200 uL of buffer AL and 20 uL of proteinase K, and vortex the tube for 15 secs to mix thoroughly.**
3. **Incubate the tube at 56˚C until the sample is completely lysed.**
   - *For efficient lysis, vortex the tube every 30 mins during the incubation. Use of a specialized instrument such as thermo-mixer will accelerate the lysis.*
4. **Add 200 uL of buffer BL to the tube and vortex for 15 secs to mix thoroughly.**
5. **Incubate the tube at 70˚C for 10 mins.**
6. **Add 200 uL of absolute ethanol to the tube, vortex twice for 5 secs to mix.**
7. **Go to 'DNA Purification procedure'.**

I. Urine

1. **Transfer up to 1 mL of urine to a 1.5 mL tube.**
2. **Centrifuge for 2 mins and discard the supernatant.**
3. **Add 200 uL of 1x PBS and vortex the tube vigorously for 15 secs to resuspend and wash the pellet.**
4. **Centrifuge for 2 mins, discard the supernatant, and resuspend the pellet with the remaining solution.**
5. **Add 200 uL of buffer AL and 20 uL of proteinase K, and vortex the tube for 15 secs to mix thoroughly.**
   - *Add 20 uL of 1M DTT if the sperm cells which can be contained in urine are the target of application.*

6. **Incubate the tube at 56˚C for 1 hour.**
    - *For efficient lysis, vortex the tube every 10 mins during the incubation. Use of a specialized instrument such as thermo-mixer will accelerate the lysis.*
7. **Add 200 uL of buffer BL to the tube and vortex for 15 secs to mix thoroughly.**
8. **Incubate the tube at 70˚C for 10 mins.**
9. **Add 200 uL of absolute ethanol to the tube, vortex twice for 5 secs to mix.**
10. **Go to 'DNA Purification procedure'.**

J. Chewing gum

1. **Cut up to 30 mg of chewing gum into small pieces and place into 1.5 mL tube.**
    - Wear gloves and use sterile blade or scalpel.
2. **Add 300 uL of buffer AL and 20 uL of proteinase K and vortex the tube for 15 secs to mix.**
3. **Incubate the tube at 56˚C for 3 hours.**
    - *For efficient lysis, vortex the tube every 30 mins during the incubation. Use of a specialized instrument such as thermo-mixer will accelerate the lysis.*
4. **Add 300 uL of buffer BL to the tube and vortex for 15 secs to mix thoroughly.**
5. **Incubate the tube at 70˚C for 10 mins.**
6. **Add 300 uL of absolute ethanol to the tube, vortex twice for 5 secs to mix.**
7. **Go to 'DNA Purification procedure'**

DNA Purification Procedure

1. **Transfer up to 700 uL of the mixture into a spin column, centrifuge for 1 min, empty the collecting tube and re-insert the spin column back to the tube.**
   - *If the mixture remains, repeat this step with the remaining mixture.*
2. **Apply 500 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.**
3. **Apply 750 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.**
4. **Centrifuge the tube for 3 mins 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.*
5. **Apply 20 ~ 50 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 20 uL, especially when the expected yield is low. The eluent volume should not be less than 20 uL, because lesser volume will not soak the membrane entirely and this lead to loss of DNA.*
6. **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 very low population of cells. Use of the carrier RNA with 1 ug per sample will help DNA bind to spin column membrane, especially when the DNA mass is very low. The carrier RNA can be applied to the lysate or buffer AL. Reduce the elution volume to minimum. The eluent volume can be decreased to 20 uL.
	Inefficient lysis	Some samples take a long time to be completely lysed. Vortex occasionally the lysate tube during incubation. Using the specialized instrument such as thermo-mixer can greatly accelerate the lysis. 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.
	Column expired or mis-placed	Micro spin column should be stored under 4℃ avoiding direct sunlight. Make sure the storage condition.
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	In case of DNA preparation from a certain animal blood or from an improperly stored blood, it is

	of hemoglobin	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 Purificaition Procedure'.
Clogging of spin column	*Inefficient lysis*	Small particulates in incomplete lysate can clog the pore of the filter membrane. If any particulates or debris still remain after lysis, centrifuge for 1 min and use the supernatant for next step. Please refer to the recommendation for inefficient lysis above.
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 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.