Ver 2.0

# Handbook for
## DNA micro


TOTAL DNA PURIFICATION KIT

## Customer & Technical Support

Should you have any further questions, do not hesitate to contact us.

We appreciate your comments and advice.

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This protocol handbook is included in :

GeneAll® Exgene™ Genomic DNA micro (118-050)

Visit www.geneall.com for FAQ, Q&A and more information.

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| Exgene™ Genomic DNA micro | | |
|---|---|---|
| **Cat. No.** | **118-050** | |
| **Components** | **Quantity** | **Storage** |
| **Size** | **micro** | |
| No. of preparation | 50 | Room temperature (15~25°C) |
| Column Type Micro S (with collection tube) | 50 | |
| Collection tube | 100 | |
| Buffer CL | 25 ml | |
| Buffer BL | 25 ml | |
| Buffer BW (concentrate) * | 30 ml | |
| Buffer TW (concentrate) * | 50 ml | |
| Buffer AE ** | 15 ml | |
| Carrier RNA | 60 μg | |
| Proteinase K † | 24 mg | |
| PK Storage buffer † | 1.2 ml | |
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** Before the first use, add the appropriate amount of absolute ethanol (ACS grade quality or higher) to Buffer BW, TW as indicated on the bottle.*

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

*† Proteinase K solution is stable at 4°C. For long term stability, store it at -20°C.*
*Refer to the instruction of storage condition on page 5.*

## Precautions and Disclaimer

Exgene™ Genomic DNA micro kit is for research use only, and should not be used for drug, household or other unintended uses. All due care and attention should be taken in every procedure in this handbook. Please consult the Material Safety Data Sheet (MSDS) for information regarding hazard and safe handling practices.

## Storage Condition

All components of Exgene™ Genomic DNA micro kit should be stored at room temperature (15~25°C). After reconstitution of Proteinase K with Storage buffer, it should be stored under 4°C for conservation of activity. It can be stored at 4°C for 1 year without significant decrease in activity. But for prolonged preservation of activity, storing under -20°C is recommended.

Under cool ambient condition, a precipitate can be formed in Buffer CL and/or BL. In such a case, heat the bottle above 56°C to dissolve completely. Exgene™ Genomic DNA micro kit is guaranteed until the expiration date printed on the product label.

## Quality Control

All components in Exgene™ Genomic DNA micro kit is manufactured in strictly clean condition, and its degree of cleanness is monitored periodically. Restriction enzyme assay, PCR amplification assay and spectrophotometric assay as quality control are carried out from lot-to-lot thoroughly, and only the qualified is approved to be delivered.

## Safety Information

Buffer BL and BW contain irritant which is harmful when in contact with skin or eyes, or when inhaled or swallowed. Care should be taken during handling. Always wear gloves and eye protector, and follow standard safety precautions.

## General handling

When working with small-size samples, microbiological aseptic technique should be always used for prevention of contamination by microbe and other contaminants.

Always wear disposable gloves while handling reagents and samples. Also, we recommend the use of sterile tip, tube and other instruments.

## Carrier RNA

This kit is provided with Carrier RNA, which can be added to Buffer BL if required. Carrier RNA enhances binding of DNA to the micro column membrane, especially if there are very few target molecules in the sample.

For purification of DNA from very small amounts of sample, we recommend adding Carrier RNA to Buffer BL. To obtain a solution of 1 μg/μl, add 60 μl of Buffer AE to the tube containing 60 μg lyophilized Carrier RNA. Dissolve the Carrier RNA thoroughly, divide it into conveniently sized aliquots, and store at -20°C. Don't freeze-thaw the aliquots of Carrier RNA more than 3 times. For one DNA preparation, 1 μl of dissolved Carrier RNA is required.

## Additional equipments or materials to be supplied by user

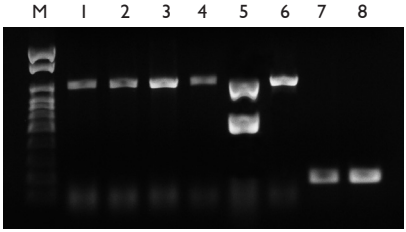
- Sterile microcentrifuge tube
- Centrifuge
- Water bath or heating block
- Ethanol (99~100%)
- 1 M Dithiothreitol (DTT)
- SPEX Freezer Mill® or metal blender for bones and teeth
- Other general lab equipments

# Product Description

The Exgene™ Genomic DNA micro kit provides fast and easy methods for the micro scale purification of total (genomic and mitochondrial) DNA from various biological samples. Purified DNA can be used directly for PCR, quantitative PCR, genotyping such as STR analysis and other downstream applications.

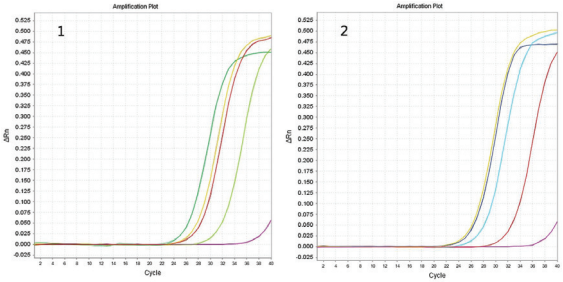
Exgene™ Genomic DNA micro kit utilizes the advanced silica-binding technology to purify total DNA sufficiently pure for many applications. Various samples are lysed in optimized buffer containing detergents and lytic enzyme. Under high salt condition, DNA in the lysate bind to silica membrane and impurities pass through membrane into a collection tube. The membranes are washed with a series of alcohol-containing buffer to remove any traces of proteins, cellular debris and salts. Finally pure DNA is released into a clean collection tube with deionized water or low ionic strength buffer.

## PCR Amplification




PCR reaction was performed with purified DNA using Exgene™ Genomic DNA micro kit. Template was isolated from whole blood (Lane 1), dried blood spot (Lane 2), hair root (Lane 3), chewing gum (Lane 4), animal tissue (Lane 5), urine (Lane 6), bone (Lane 7) and hair shaft (Lane 8).
M : 1 Kb ladder

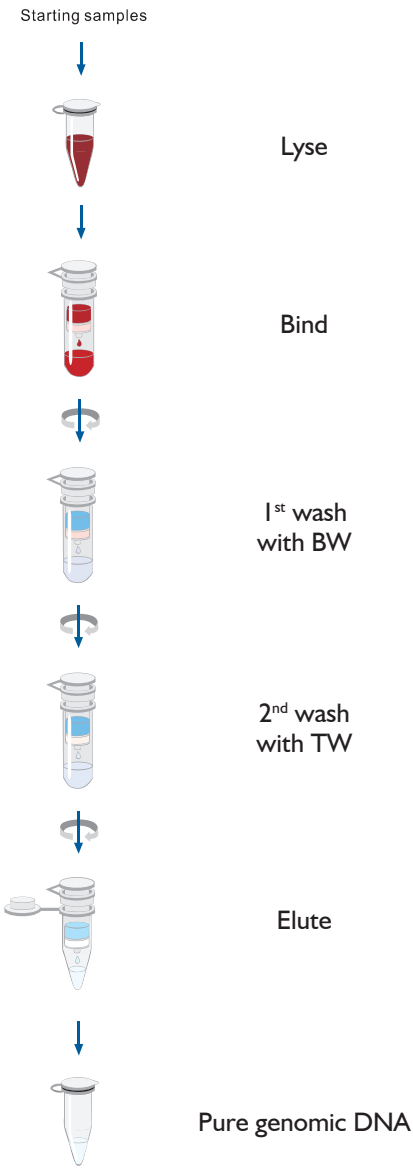
## Real-time PCR Amplification




Real-time PCR was performed with purified DNA using Exgene™ Genomic DNA micro kit. The DNA was extracted from whole blood, stains, swab and hair root (Panel 1), nail clippings, chewing gum, tooth brush and urine (Panel 2). Real-time PCR was carried out with human GAPDH primer sets, and detected by SYBR® Green.

# KIT PROCEDURES

in microcentrifuges



# A.
# PROTOCOL FOR.
# Small Volumes of Blood



***Before experiment***

*Prepare the water bath 56°C*
*Prepare absolute ethanol*
*Prepare 1.5 ml microcentrifuge tube*
*Equilibrate Buffer AE to room temperature*
*All centrifugation should be performed at room temperature*
*Buffer BL and CL may precipitate at cool ambient temperature If so, dissolve it in 56°C water bath*

1. **Pipet 10 μl of Proteinase K solution into the bottom of a 1.5 ml microcentrifuge tube.**

2. **Transfer 1~100 μl of whole blood to the tube.**
   If the whole blood volume is less than 100 μl, adjust the volume to 100 μl with Buffer CL.

3. **Add 100 μl of Buffer BL to the tube. Vortex the tube to mix thoroughly. Incubate the tube at 56°C for 10 min. Spin down briefly to remove any drops from inside of the lid.**
   It is essential to mix the sample and Buffer BL thoroughly for good result.
   If the volume of blood is lower than 10 μl, recommend adding Carrier RNA to Buffer BL.

4. **Add 100 μl of absolute ethanol (not provided) to the sample, pulse vortex to mix the sample thoroughly, and spin down briefly to remove any drops from inside of the lid.**
   It is important to mix the sample and ethanol completely for good result.

5. **Transfer the mixture to the column carefully, centrifuge for 1 min at 6,000 x g above (>8,000 rpm), and replace the collection tube with new one (provided).**
   If the mixture has not passed completely through the membrane, centrifuge again at full speed (> 13,000 x g) until all of the solution has passed through.
   Centrifugation at full speed will not affect DNA recovery.

6. **Add 500 µl of Buffer BW, centrifuge for 1 min at 6,000 x g above (>8,000 rpm) and replace the collection tube with new one (provided).**
   Centrifugation at full speed will not affect DNA recovery.

7. **Apply 700 µl of Buffer TW. Centrifuge for 1 min at 6,000 x g above (>8,000 rpm). Discard the pass-through and reinsert the column back into the collection tube.**
   Centrifugation at full speed will not affect DNA recovery.

8. **Centrifuge at full speed for 3 min to remove residual wash buffer. Place the column in a fresh 1.5 ml tube (not provided).**
   Care must be taken at this step for eliminating the carryover of Buffer TW. If carryover of Buffer TW still occurs, centrifuge again for 3 min at full speed with the collection tube before transferring to a new 1.5 ml tube. Centrifugation must be performed at full speed (13,000~20,000 x g).

9. **Add 20~50 µl of Buffer AE or sterilized water. Incubate for 1 min at room temperature. Centrifuge at full speed for 1 min.**
   Ensure that the Buffer AE or sterilized water is dispensed directly onto the center of column membrane for optimal elution of DNA.

# B.
# PROTOCOL FOR.
## Swab (blood, saliva or sperm)



***Before experiment***

*Prepare the water bath 56°C*
*Prepare absolute ethanol*
*Prepare 1.5 ml microcentrifuge tube*
*Equilibrate Buffer AE to room temperature*
*All centrifugation should be performed at room temperature*
*Buffer BL and CL may precipitate at cool ambient temperature If so, dissolve it in 56°C water bath*

1. **Place the swab in a 1.5 ml microcentrifuge tube.**

2. **Add 300 μl of Buffer CL and 20 μl of Proteinase K. Vortex the tube to mix thoroughly. Incubate the tube at 56°C for 1 h. Spin down briefly to remove any drops from inside of the lid.**
   If processing semen swab, add 20 μl of 1 M DTT as well.
   For efficient lysis, mix the sample completely. To help the efficient lysis, vortex the tube every 10 min during the incubation.

3. **Add 300 μl of Buffer BL to the tube. Vortex the tube to mix thoroughly. Spin down briefly to remove any drops from inside of the lid.**
   It is essential to mix the sample and Buffer BL thoroughly for good result.
   If Carrier RNA is required, add the dissolved Carrier RNA to Buffer BL.

4. **Add 300 μl of absolute ethanol (not provided) to the sample, pulse vortex to mix the sample thoroughly, and spin down briefly to remove any drops from inside of the lid.**
   It is important to mix the sample and ethanol completely for good result.

5. **Transfer the mixture to the column carefully, centrifuge for 1 min at 6,000 x g above (>8,000 rpm), and replace the collection tube with new one (provided).**
   If the mixture is remained, apply the mixture twice; apply 700 μl of the mixture, spin down, discard the pass-through, re-insert empty collection tube, and repeat this step again until all of the mixture has applied to the micro column.
   If the mixture has not passed completely through the membrane, centrifuge again at full speed (> 13,000 x g) until all of the solution has passed through.
   Centrifugation at full speed will not affect DNA recovery.

6. **Add 600 μl of Buffer BW, centrifuge for 1 min at 6,000 x g above (>8,000 rpm) and replace the collection tube with new one (provided).**
   Centrifugation at full speed will not affect DNA recovery.

7. **Apply 700 μl of Buffer TW. Centrifuge for 1 min at 6,000 x g above (>8,000 rpm). Discard the pass-through and reinsert the column back into the collection tube.**
   Centrifugation at full speed will not affect DNA recovery.

8. **Centrifuge at full speed for 3 min to remove residual wash buffer. Place the column in a fresh 1.5 ml tube (not provided).**
   Care must be taken at this step for eliminating the carryover of Buffer TW. If carryover of Buffer TW still occurs, centrifuge again for 3 min at full speed with the collection tube before transferring to a new 1.5 ml tube. Centrifugation must be performed at full speed (13,000~20,000 x g).

9. **Add 20~50 μl of Buffer AE or sterilized water. Incubate for 1 min at room temperature. Centrifuge at full speed for 1 min.**
   Ensure that the Buffer AE or sterilized water is dispensed directly onto the center of column membrane for optimal elution of DNA.

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

***Before experiment***

*Prepare the water bath 56°C*
*Prepare absolute ethanol*
*Prepare 1.5 ml microcentrifuge tube*
*Equilibrate Buffer AE to room temperature*
*All centrifugation should be performed at room temperature*
*Buffer BL and CL may precipitate at cool ambient temperature If so, dissolve it in 56°C water bath*

1. **Place 0.5 $cm^2$ of punched-out circles from stained materials into a 1.5 ml microcentrifuge tube.**

2. **Add 200 µl of Buffer CL and 20 µl of Proteinase K. Vortex the tube to mix thoroughly. Incubate the tube at 56°C for 1 h. Spin down briefly to remove any drops from inside of the lid.**
   If processing semen stains, add 20 µl of 1 M DTT as well.
   For efficient lysis, mix the sample completely. To help the efficient lysis, vortex the tube every 10 min during the incubation.

3. **Add 200 µl of Buffer BL to the tube. Vortex the tube to mix thoroughly. Spin down briefly to remove any drops from inside of the lid.**
   It is essential to mix the sample and Buffer BL thoroughly for good result.
   If Carrier RNA is required, add the dissolved Carrier RNA to Buffer BL.

4. **Add 200 µl of absolute ethanol (not provided) to the sample, pulse vortex to mix the sample thoroughly, and spin down briefly to remove any drops from inside of the lid.**
   It is important to mix the sample and ethanol completely for good result.

5. **Transfer the supernatant to the column carefully, centrifuge for 1 min at 6,000 x g above (>8,000 rpm), and replace the collection tube with new one (provided).**
   If the mixture has not passed completely through the membrane, centrifuge again at full speed (> 13,000 x g) until all of the solution has passed through.
   Centrifugation at full speed will not affect DNA recovery.

6. **Add 600 µl of Buffer BW, centrifuge for 1 min at 6,000 x g above (>8,000 rpm) and replace the collection tube with new one (provided).**
   Centrifugation at full speed will not affect DNA recovery.

7. **Apply 700 µl of Buffer TW. Centrifuge for 1 min at 6,000 x g above (>8,000 rpm). Discard the pass-through and reinsert the column back into the collection tube.**
   Centrifugation at full speed will not affect DNA recovery.

8. **Centrifuge at full speed for 3 min to remove residual wash buffer. Place the column in a fresh 1.5 ml tube (not provided).**
   Care must be taken at this step for eliminating the carryover of Buffer TW. If carryover of Buffer TW still occurs, centrifuge again for 3 min at full speed with the collection tube before transferring to a new 1.5 ml tube. Centrifugation must be performed at full speed (13,000~20,000 x g).

9. **Add 20~50 µl of Buffer AE or sterilized water. Incubate for 1 min at room temperature. Centrifuge at full speed for 1 min.**
   Ensure that the Buffer AE or sterilized water is dispensed directly onto the center of column membrane for optimal elution of DNA.

# D.
# PROTOCOL FOR.
# Hair and Nail Clippings

***Before experiment***

*Prepare the water bath 56°C*
*Prepare absolute ethanol*
*Prepare 1.5 ml microcentrifuge tube*
*Equilibrate Buffer AE to room temperature*
*All centrifugation should be performed at room temperature*
*Buffer BL and CL may precipitate at cool ambient temperature If so, dissolve it in 56°C water bath*

1. **Collect hair (root or shaft) or nail clippings sample in a 1.5 ml microcentrifuge tube.**

2. **Add 200 μl of Buffer CL, 20 μl of Proteinase K and 20 μl of 1 M DTT, vortex to mix and incubate the tube at 56°C for at least 1 h until the sample is dissolved. Spin down briefly to remove any drops from inside of the lid.**
   For efficient lysis, mix the sample completely. To help the efficient lysis, vortex the tube every 10 min during the incubation.
   For nail clippings, it is recommended to incubate overnight incubation at 56°C.

3. **Add 200 μl of Buffer BL to the tube. Vortex the tube to mix thoroughly. Spin down briefly to remove any drops from inside of the lid.**
   It is essential to mix the sample and Buffer BL thoroughly for good result.
   If Carrier RNA is required, add the dissolved Carrier RNA to Buffer BL.

4. **Add 200 μl of absolute ethanol (not provided) to the sample, pulse vortex to mix the sample thoroughly, and spin down briefly to remove any drops from inside of the lid.**
   It is important to mix the sample and ethanol completely for good result.

5. **Transfer the supernatant to the column carefully, centrifuge for 1 min at 6,000 x g above (>8,000 rpm), and replace the collection tube with new one (provided).**
   If the mixture has not passed completely through the membrane, centrifuge again at full speed (>13,000 x g) until all of the solution has passed through.
   Centrifugation at full speed will not affect DNA recovery.

6. **Add 600 μl of Buffer BW, centrifuge for 1 min at 6,000 x g above (>8,000 rpm) and replace the collection tube with new one (provided).**
   Centrifugation at full speed will not affect DNA recovery.

7. **Apply 700 μl of Buffer TW. Centrifuge for 1 min at 6,000 x g above (>8,000 rpm). Discard the pass-through and reinsert the column back into the collection tube.**
   Centrifugation at full speed will not affect DNA recovery.

8. **Centrifuge at full speed for 3 min to remove residual wash buffer. Place the column in a fresh 1.5 ml tube (not provided).**
   Care must be taken at this step for eliminating the carryover of Buffer TW. If carryover of Buffer TW still occurs, centrifuge again for 3 min at full speed with the collection tube before transferring to a new 1.5 ml tube. Centrifugation must be performed at full speed (13,000~20,000 x g).

9. **Add 20~50 μl of Buffer AE or sterilized water. Incubate for 1 min at room temperature. Centrifuge at full speed for 1 min.**
   Ensure that the Buffer AE or sterilized water is dispensed directly onto the center of column membrane for optimal elution of DNA.

# E.
# PROTOCOL FOR.
# Bones and Teeth

***Before experiment***

*Prepare the water bath 56°C*
*Prepare absolute ethanol*
*Prepare 1.5 ml microcentrifuge tube*
*Equilibrate Buffer AE to room temperature*
*All centrifugation should be performed at room temperature*
*Buffer BL and CL may precipitate at cool ambient temperature If so, dissolve it in 56°C water bath*

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

**- The use of the 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.

**- The use of 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.

**- The use of EDTA**

Transfer the bones or teeth into centrifuge tube. Pour the 0.5 M EDTA 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 occasionally during incubation. Cut the sample as small as possible with a microtome or blade.

2. **Place up to 100 mg of bones or teeth into a 1.5 ml microcentrifuge tube.**

3. **Add 300 µl of Buffer CL and 20 µl of Proteinase K, vortex to mix. Incubate overnight at 56°C. Spin down briefly to remove any drops from inside of the lid.**
   For efficient lysis, recommend to lyse using rotator within incubator.

4. **Add 300 µl of Buffer BL to the tube. Vortex the tube to mix thoroughly. Spin down briefly to remove any drops from inside of the lid.**
   It is essential to mix the sample and Buffer BL thoroughly for good result.
   If Carrier RNA is required, add the dissolved Carrier RNA to Buffer BL.

5. **Centrifuge the tube at full speed for 1 min, and carefully transfer the supernatant to a new 1.5 ml microcentrifuge tube.**

6. **Add 300 µl of absolute ethanol (not provided) to the sample, pulse vortex to mix the sample thoroughly, and spin down briefly to remove any drops from inside of the lid.**
   It is important to mix the sample and ethanol completely for good result.

7. **Transfer the mixture to the column carefully, centrifuge for 1 min at 6,000 x g above (>8,000 rpm), and replace the collection tube with new one (provided).**
   If the mixture is remained, apply the mixture twice; apply 700 µl of the mixture, spin down, discard the pass-through, re-insert empty collection tube, and repeat this step again until all of the mixture has applied to the micro column.
   If the mixture has not passed completely through the membrane, centrifuge again at full speed (> 13,000 x g) until all of the solution has passed through.
   Centrifugation at full speed will not affect DNA recovery.

8. **Add 600 µl of Buffer BW, centrifuge for 1 min at 6,000 x g above (>8,000 rpm) and replace the collection tube with new one (provided).**
   Centrifugation at full speed will not affect DNA recovery.

9. **Apply 700 μl of Buffer TW. Centrifuge for 1 min at 6,000 x g above (>8,000 rpm). Discard the pass-through and reinsert the column back into the collection tube.**
   Centrifugation at full speed will not affect DNA recovery.

10. **Centrifuge at full speed for 3 min to remove residual wash buffer. Place the column in a fresh 1.5 ml tube (not provided).**
    Care must be taken at this step for eliminating the carryover of Buffer TW. If carryover of Buffer TW still occurs, centrifuge again for 3 min at full speed with the collection tube before transferring to a new 1.5 ml tube. Centrifugation must be performed at full speed (13,000~20,000 x g).

11. **Add 20~50 μl of Buffer AE or sterilized water. Incubate for 1 min at room temperature. Centrifuge at full speed for 1 min.**
    Ensure that the Buffer AE or sterilized water is dispensed directly onto the center of column membrane for optimal elution of DNA.

# F.
# PROTOCOL FOR.
# Cigarette Butts

***Before experiment***

*Prepare the water bath 56°C and 70°C*
*Prepare absolute ethanol*
*Prepare 1.5 ml microcentrifuge tube*
*Equilibrate Buffer AE to room temperature*
*All centrifugation should be performed at room temperature*
*Buffer BL and CL may precipitate at cool ambient temperature If so, dissolve it in 56°C water bath*

1. **Cut out a 1 cm² piece of outer paper from the end of the cigarette or filter. Cut this piece into 6 smaller pieces. Transfer the pieces to a 1.5 ml microcentrifuge tube.**
   Wear gloves and use sterile scissors or scalpel.

2. **Add 300 µl of Buffer CL and 20 µl of Proteinase K, vortex to mix. Incubate the tube at 56°C for 1 h. Spin down briefly to remove any drops from inside of the lid.**
   For efficient lysis, mix the sample completely. To help the efficient lysis, vortex the tube every 10 min during the incubation.

3. **Add 300 µl of Buffer BL to the tube. Vortex the tube to mix thoroughly. Incubate the tube at 70°C for 10 min. Spin down briefly to remove any drops from inside of the lid.**
   It is essential to mix the sample and Buffer BL thoroughly for good result.
   If Carrier RNA is required, add the dissolved Carrier RNA to Buffer BL.

4. **Add 300 µl of absolute ethanol (not provided) to the sample, pulse vortex to mix the sample thoroughly, and spin down briefly to remove any drops from inside of the lid.**
   It is important to mix the sample and ethanol completely for good result.

5. **Transfer the supernatant to the column carefully, centrifuge for 1 min at 6,000 x g above (>8,000 rpm), and replace the collection tube with new one (provided).**
   If the mixture is remained, apply the mixture twice; apply 700 μl of the mixture, spin down, discard the pass-through, re-insert empty collection tube, and repeat this step again until all of the mixture has applied to the micro column.
   If the mixture has not passed completely through the membrane, centrifuge again at full speed (> 13,000 x g) until all of the solution has passed through.
   Centrifugation at full speed will not affect DNA recovery.

6. **Add 500 μl of Buffer BW, centrifuge for 1 min at 6,000 x g above (>8,000 rpm) and replace the collection tube with new one (provided).**
   Centrifugation at full speed will not affect DNA recovery.

7. **Apply 700 μl of Buffer TW. Centrifuge for 1 min at 6,000 x g above (>8,000 rpm). Discard the pass-through and reinsert the column back into the collection tube.**
   Centrifugation at full speed will not affect DNA recovery.

8. **Centrifuge at full speed for 3 min to remove residual wash buffer. Place the column in a fresh 1.5 ml tube (not provided).**
   Care must be taken at this step for eliminating the carryover of Buffer TW. If carryover of Buffer TW still occurs, centrifuge again for 3 min at full speed with the collection tube before transferring to a new 1.5 ml tube. Centrifugation must be performed at full speed (13,000~20,000 x g).

9. **Add 20~50 μl of Buffer AE or sterilized water. Incubate for 1 min at room temperature. Centrifuge at full speed for 1 min.**
   Ensure that the Buffer AE or sterilized water is dispensed directly onto the center of column membrane for optimal elution of DNA.

# G.
# PROTOCOL FOR.
# Tooth Brush

***Before experiment***

*Prepare the water bath 56°C and 70°C*
*Prepare absolute ethanol*
*Prepare 1.5 ml microcentrifuge tube*
*Equilibrate Buffer AE to room temperature*
*All centrifugation should be performed at room temperature*
*Buffer BL and CL may precipitate at cool ambient temperature If so, dissolve it in 56°C water bath*

1. **Collect bristles on tooth brush in a 1.5 ml microcentrifuge tube.**
   Alternatively, rinse the tooth brush with 10 ml of 1 X PBS. Collect the buccal cells by centrifugation.

2. **Add 200 µl of Buffer CL and 20 µl of Proteinase K, vortex to mix. Incubate the tube at 56°C for 1 h. Spin down briefly to remove any drops from inside of the lid.**
   For efficient lysis, mix the sample completely. To help the efficient lysis, vortex the tube every 10 min during the incubation.

3. **Add 200 µl of Buffer BL to the tube. Vortex the tube to mix thoroughly. Incubate the tube at 70°C for 10 min. Spin down briefly to remove any drops from inside of the lid.**
   It is essential to mix the sample and Buffer BL thoroughly for good result.
   If Carrier RNA is required, add the dissolved Carrier RNA to Buffer BL.

4. **Add 200 µl of absolute ethanol (not provided) to the sample, pulse vortex to mix the sample thoroughly, and spin down briefly to remove any drops from inside of the lid.**
   It is important to mix the sample and ethanol completely for good result.

5. **Transfer the supernatant to the column carefully, centrifuge for 1 min at 6,000 x g above (>8,000 rpm), and replace the collection tube with new one (provided).**
   If the mixture has not passed completely through the membrane, centrifuge again at full speed (> 13,000 x g) until all of the solution has passed through.
   Centrifugation at full speed will not affect DNA recovery.

6. **Add 500 µl of Buffer BW, centrifuge for 1 min at 6,000 x g above (>8,000 rpm) and replace the collection tube with new one (provided).**
   Centrifugation at full speed will not affect DNA recovery.

7. **Apply 700 µl of Buffer TW. Centrifuge for 1 min at 6,000 x g above (>8,000 rpm). Discard the pass-through and reinsert the column back into the collection tube.**
   Centrifugation at full speed will not affect DNA recovery.

8. **Centrifuge at full speed for 3 min to remove residual wash buffer. Place the column in a fresh 1.5 ml tube (not provided).**
   Care must be taken at this step for eliminating the carryover of Buffer TW. If carryover of Buffer TW still occurs, centrifuge again for 3 min at full speed with the collection tube before transferring to a new 1.5 ml tube. Centrifugation must be performed at full speed (13,000~20,000 x g).

9. **Add 20~50 µl of Buffer AE or sterilized water. Incubate for 1 min at room temperature. Centrifuge at full speed for 1 min.**
   Ensure that the Buffer AE or sterilized water is dispensed directly onto the center of column membrane for optimal elution of DNA.

# H.
# PROTOCOL FOR.
## Tissue

***Before experiment***

*Prepare the water bath 56°C*
*Prepare absolute ethanol*
*Prepare 1.5 ml microcentrifuge tube*
*Equilibrate Buffer AE to room temperature*
*All centrifugation should be performed at room temperature*
*Buffer BL and CL may precipitate at cool ambient temperature If so, dissolve it in 56°C water bath*

1. **Transfer less than 10 mg of a tissue sample to a 1.5 ml microcentrifuge tube.**

2. **Add 200 μl of Buffer CL and 20 μl of Proteinase K. Mix completely by vortexing or pipetting. Incubate the tube at 56°C until the sample is completely lysed. Spin down briefly to remove any drops from inside of the lid.**
   It is essential to mix the components completely for proper lysis. The lysate should become translucent without any particles after complete lysis.
   To help the efficient lysis, vortex the tube occasionally (2~3 times per hour) during the incubation.

3. **Add 200 μl of Buffer BL to the tube. Vortex the tube to mix thoroughly. Spin down briefly to remove any drops from inside of the lid.**
   It is essential to mix the sample and Buffer BL thoroughly for good result.
   If Carrier RNA is required, add the dissolved Carrier RNA to Buffer BL.

4. **Add 200 µl of absolute ethanol (not provided) to the sample, pulse vortex to mix the sample thoroughly, and spin down briefly to remove any drops from inside of the lid.**
   It is important to mix the sample and ethanol completely for good result.

5. **Transfer the mixture to the column carefully, centrifuge for 2 min at 6,000 x g above (>8,000 rpm), and replace the collection tube with new one (provided).**
   If the mixture has not passed completely through the membrane, centrifuge again at full speed (>13,000 x g) until all of the solution has passed through.
   Centrifugation at full speed will not affect DNA recovery.

6. **Add 600 µl of Buffer BW, centrifuge for 2 min at 6,000 x g above (>8,000 rpm) and replace the collection tube with new one (provided).**
   Centrifugation at full speed will not affect DNA recovery.

7. **Apply 700 µl of Buffer TW. Centrifuge for 2 min at 6,000 x g above (>8,000 rpm). Discard the pass-through and reinsert the column back into the collection tube.**
   Centrifugation at full speed will not affect DNA recovery.

8. **Centrifuge at full speed for 3 min to remove residual wash buffer. Place the column in a fresh 1.5 ml tube (not provided).**
   Care must be taken at this step for eliminating the carryover of Buffer TW. If carryover of Buffer TW still occurs, centrifuge again for 3 min at full speed with the collection tube before transferring to a new 1.5 ml tube. Centrifugation must be performed at full speed (13,000~20,000 x g).

9. **Add 20~50 µl of Buffer AE or sterilized water. Incubate for 1 min at room temperature. Centrifuge at full speed for 1 min.**
   Ensure that the Buffer AE or sterilized water is dispensed directly onto the center of column membrane for optimal elution of DNA.

# I.
# PROTOCOL FOR.
# Urine

***Before experiment***

*Prepare the water bath 56°C*
*Prepare absolute ethanol*
*Prepare 1.5 ml microcentrifuge tube*
*Equilibrate Buffer AE to room temperature*
*All centrifugation should be performed at room temperature*
*Buffer BL and CL may precipitate at cool ambient temperature If so, dissolve it in 56°C water bath*

1. **Transfer up to 1 ml urine to a 1.5 ml microcentrifuge tube and centrifuge for 2 min at 6,000 x g above (>8,000 rpm).**

2. **Discard the supernatant. Add 200 µl of 1 X PBS then vortex the tube for 5 sec.**

3. **Centrifuge for 2 min at 6,000 x g above (>8,000 rpm). Then discard the supernatant.**

4. **Add 200 µl of Buffer CL and 20 µl of Proteinase K. Vortex to mix. Incubate the tube at 56°C for 1 h. Spin down briefly to remove any drops from inside of the lid.**
   Since urine can contain sperm cells, add 20 µl of 1 M DTT as well.
   For efficient lysis, mix the sample completely. To help the efficient lysis, vortex the tube every 10 min during the incubation.

5. **Add 200 μl of Buffer BL and 200 μl of absolute ethanol (not provided) to the tube. Vortex the tube to mix thoroughly. Spin down briefly to remove any drops from inside of the lid.**
   It is important to mix the sample, Buffer BL and ethanol completely for good result. If Carrier RNA is required, add the dissolved Carrier RNA to Buffer BL.

6. **Transfer the mixture to the column carefully, centrifuge for 1 min at 6,000 x g above (>8,000 rpm), and replace the collection tube with new one (provided).**
   If the mixture has not passed completely through the membrane, centrifuge again at full speed (> 13,000 x g) until all of the solution has passed through.
   Centrifugation at full speed will not affect DNA recovery.

7. **Add 500 μl of Buffer BW, centrifuge for 1 min at 6,000 x g above (>8,000 rpm) and replace the collection tube with new one (provided).**
   Centrifugation at full speed will not affect DNA recovery.

8. **Apply 700 μl of Buffer TW. Centrifuge for 1 min at 6,000 x g above (>8,000 rpm). Discard the pass-through and reinsert the column back into the collection tube.**
   Centrifugation at full speed will not affect DNA recovery.

9. **Centrifuge at full speed for 3 min to remove residual wash buffer. Place the column in a fresh 1.5 ml tube (not provided).**
   Care must be taken at this step for eliminating the carryover of Buffer TW. If carryover of Buffer TW still occurs, centrifuge again for 3 min at full speed with the collection tube before transferring to a new 1.5 ml tube. Centrifugation must be performed at full speed (13,000~20,000 x g).

10. **Add 20~50 μl of Buffer AE or sterilized water. Incubate for 1 min at room temperature. Centrifuge at full speed for 1 min.**
    Ensure that the Buffer AE or sterilized water is dispensed directly onto the center of column membrane for optimal elution of DNA.

# J.
# PROTOCOL FOR.
# Chewing Gum

***Before experiment***

*Prepare the water bath 56°C and 70°C*
*Prepare absolute ethanol*
*Prepare 1.5 ml microcentrifuge tube*
*Equilibrate Buffer AE to room temperature*
*All centrifugation should be performed at room temperature*
*Buffer BL and CL may precipitate at cool ambient temperature If so, dissolve it in 56°C water bath*

1. **Cut up to 30 mg of chewing gum into small pieces and them to a 1.5 ml microcentrifuge tube.**
   Wear gloves and use sterile blade or scalpel.

2. **Add 300 µl of Buffer CL and 20 µl of Proteinase K. Mix completely by vortexing or pipetting. Incubate the tube at 56°C for 3 h. Spin down briefly to remove any drops from inside of the lid.**
   For efficient lysis, mix the sample completely. To help the efficient lysis, vortex the tube every 30 min during the incubation.

3. **Add 300 µl of Buffer BL to the tube. Vortex the tube to mix thoroughly. Incubate the tube at 70°C for 10 min. Spin down briefly to remove any drops from inside of the lid.**
   It is essential to mix the sample and Buffer BL thoroughly for good result.
   If Carrier RNA is required, add the dissolved Carrier RNA to Buffer BL.

4. **Add 300 µl of absolute ethanol (not provided) to the sample, pulse vortex to mix the sample thoroughly, and spin down briefly to remove any drops from inside of the lid.**
   It is important to mix the sample and ethanol completely for good result.

5. **Transfer the supernatant to the column carefully, centrifuge for 1 min at 6,000 x g above (>8,000 rpm), and replace the collection tube with new one (provided).**
   If the mixture remain, apply the mixture twice; apply 700 μl of the mixture, spin down, discard the pass-through, re-insert empty collection tube, and repeat this step again until all of the mixture has applied to the micro column.
   If the mixture has not passed completely through the membrane, centrifuge again at full speed (> 13,000 x g) until all of the solution has passed through.
   Centrifugation at full speed will not affect DNA recovery.

6. **Add 500 μl of Buffer BW, centrifuge for 1 min at 6,000 x g above (>8,000 rpm) and replace the collection tube with new one (provided).**
   Centrifugation at full speed will not affect DNA recovery.

7. **Apply 700 μl of Buffer TW. Centrifuge for 1 min at 6,000 x g above (>8,000 rpm). Discard the pass-through and reinsert the column back into the collection tube.**
   Centrifugation at full speed will not affect DNA recovery.

8. **Centrifuge at full speed for 3 min to remove residual wash buffer. Place the column in a fresh 1.5 ml tube (not provided).**
   Care must be taken at this step for eliminating the carryover of Buffer TW. If carryover of Buffer TW still occurs, centrifuge again for 3 min at full speed with the collection tube before transferring to a new 1.5 ml tube. Centrifugation must be performed at full speed (13,000~20,000 x g).

9. **Add 20~50 μl of Buffer AE or sterilized water. Incubate for 1 min at room temperature. Centrifuge at full speed for 1 min.**
   Ensure that the Buffer AE or sterilized water is dispensed directly onto the center of column membrane for optimal elution of DNA.

## Troubleshooting Guide

| Facts | Possible Causes | Suggestions |
|---|---|---|
| **Low or no yield** | **Starting material is too old or mis-stored** | Best yield will be obtained from fresh sample. DNA yield is dependent on the type, size, age and storage of starting material. Lower yield will be obtained from material that has been inappropriately stored. For example, blood samples that have been stored at 4°C for more than 5 days may bring about reduced yield. |
| | **Inefficient or insufficient lysis** | Inefficient lysis may be due to several causes;<br>- Insufficient mixing with Buffer BL<br>- Degenerated Proteinase K<br>After addition of Buffer BL in protocol, vortex the mixture vigorously and immediately to mix completely.<br>Proteinase K should be stored under 4°C for maintenance of proper activity. However, it is recommended to store in small aliquots at -20°C for prolonged preservation of its activity. |
| | **Improper eluent** | As user's need, elution buffer other than Buffer AE can be used. However, the condition of optimal elution should be low salt concentration with alkaline pH (7<pH<9). When water or other buffer was used as eluent, ensure that condition. |
| | **Cell clumps present in the lysate** | Cell clumps will remain until cells are completely lysed. Incomplete lysis of cells will bring about poor yield. To lyse completely the cells in the clumps, incubate sample at 56°C with periodic mixing until the solution is homogeneous. |

| Facts | Possible Causes | Suggestions |
|---|---|---|
| **DNA floats out of well while loading of agarose gel** | **Residual ethanol from Buffer TW remains in eluate** | Ensure that the TW wash step in protocol has been performed correctly. Micro column membrane should be completely dried via additional centrifugation or air-drying. Refer the annotation of TW washing step. |
| **Enzymatic reaction is not performed well with purified DNA** | **Residual ethanol remains in eluate** | Determine the maximum volume of eluate suitable for your amplification reaction. Reduce or increase the volume of eluate added to the amplification reaction accordingly. The elution volume can be adjusted proportionally. |
| | **High salt concentration in eluate** | Ensure that all washing steps were performed just in accordance with the protocols. Alternatively, carry out additional washing step with Buffer TW. It may help remove high salt in eluate. |
| **Column clogging** | **Inefficient lysis** | Inefficient lysis may lead to column clogging. About inefficient lysis, check 'Inefficient lysis'at "Low or no yield" |
| **Precipitate in Buffer CL or BL** | **Buffer stored in cool ambient condition** | For proper DNA purification, any precipitate in Buffer CL/BL should be dissolved by incubating the buffer at 56°C or above until it disappears. |

# Ordering Information

| Products | Scale | Size | Cat. No. | Type |
|---|---|---|---|---|
| **GeneAll® *Hybrid-Q™*** *for rapid preparation of plasmid DNA* | | | | |
| Plasmid Rapidprep | mini | 50 | 100-150 | spin |
| | | 200 | 100-102 | |
| **GeneAll® *Exprep™*** *for preparation of plasmid DNA* | | | | |
| Plasmid SV | mini | 50 | 101-150 | spin / vacuum |
| | | 200 | 101-102 | |
| | midi | 26 | 101-226 | spin / vacuum |
| | | 50 | 101-250 | |
| | | 100 | 101-201 | |
| **GeneAll® *Exfection™*** *for preparation of transfection-grade plasmid DNA* | | | | |
| Plasmid LE *(Low Endotoxin)* | mini | 50 | 111-150 | spin / vacuum |
| | | 200 | 111-102 | |
| | midi | 26 | 111-226 | spin / vacuum |
| | | 100 | 111-201 | |
| Plasmid EF *(Endotoxin Free)* | midi | 20 | 121-220 | spin |
| | | 100 | 121-201 | |
| **GeneAll® *Expin™*** *for purification of fragment DNA* | | | | |
| Gel SV | mini | 50 | 102-150 | spin / vacuum |
| | | 200 | 102-102 | |
| PCR SV | mini | 50 | 103-150 | spin / vacuum |
| | | 200 | 103-102 | |
| CleanUp SV | mini | 50 | 113-150 | spin / vacuum |
| | | 200 | 113-102 | |
| Combo GP | mini | 50 | 112-150 | spin / vacuum |
| | | 200 | 112-102 | |
| **GeneAll® *Exgene™*** *for isolation of total DNA* | | | | |
| Tissue SV | mini | 100 | 104-101 | spin / vacuum |
| | | 250 | 104-152 | |
| | midi | 26 | 104-226 | spin / vacuum |
| | | 100 | 104-201 | |
| | maxi | 10 | 104-310 | spin / vacuum |
| | | 26 | 104-326 | |
| Tissue Plus SV | mini | 100 | 109-101 | spin / vacuum |
| | | 250 | 109-152 | |
| | midi | 26 | 109-226 | spin / vacuum |
| | | 100 | 109-201 | |
| | maxi | 10 | 109-310 | spin / vacuum |
| | | 26 | 109-326 | |

| Products | Scale | Size | Cat. No. | Type |
|---|---|---|---|---|
| **GeneAll® *Exgene™*** *for isolation of total DNA* | | | | |
| Blood SV | mini | 100 | 105-101 | spin / vacuum |
| | | 250 | 105-152 | |
| | midi | 26 | 105-226 | spin / vacuum |
| | | 100 | 105-201 | |
| | maxi | 10 | 105-310 | spin / vacuum |
| | | 26 | 105-326 | |
| Cell SV | mini | 100 | 106-101 | spin / vacuum |
| | | 250 | 106-152 | |
| | maxi | 10 | 106-310 | spin / vacuum |
| | | 26 | 106-326 | |
| Clinic SV | mini | 100 | 108-101 | spin / vacuum |
| | | 250 | 108-152 | |
| | midi | 26 | 108-226 | spin / vacuum |
| | | 100 | 108-201 | |
| | maxi | 10 | 108-310 | spin / vacuum |
| | | 26 | 108-326 | |
| Genomic DNA micro | | 50 | 118-050 | spin |
| Plant SV | mini | 100 | 117-101 | spin / vacuum |
| | | 250 | 117-152 | |
| | midi | 26 | 117-226 | spin / vacuum |
| | | 100 | 117-201 | |
| | maxi | 10 | 117-310 | spin / vacuum |
| | | 26 | 117-326 | |
| Soil DNA mini | mini | 50 | 114-150 | spin |
| Stool DNA mini | mini | 50 | 115-150 | spin |
| Stool-Bead DNA mini | mini | 50 | 115-151 | spin |
| Viral DNA/RNA | mini | 50 | 128-150 | spin |
| FFPE Tissue DNA | mini | 50 | 138-150 | spin |
| | | 250 | 138-152 | |
| **GeneAll® *GenEx™*** *for isolation of total DNA without spin column* | | | | |
| *GenEx™* Blood | Sx | 100 | 220-101 | solution |
| | | 500 | 220-105 | |
| | Lx | 100 | 220-301 | solution |
| *GenEx™* Cell | Sx | 100 | 221-101 | solution |
| | | 500 | 221-105 | |
| | Lx | 100 | 221-301 | solution |
| *GenEx™* Tissue | Sx | 100 | 222-101 | solution |
| | | 500 | 222-105 | |
| | Lx | 100 | 222-301 | solution |

| Products | Scale | Size | Cat. No. | Type |
|---|---|---|---|---|
| **GeneAll® *GenEx™*** *for isolation of total DNA without spin column* | | | | |
| *GenEx*™ Plant | Sx | 100 | 227-101 | solution |
| | Mx | 100 | 227-201 | |
| | Lx | 100 | 227-301 | |
| *GenEx*™ Plant Plus | Sx | 100 | 228-101 | solution |
| | Mx | 50 | 228-250 | |
| | Lx | 20 | 228-320 | |
| **GeneAll® *DirEx™* series** *for preperation of PCR-template without extraction* | | | | |
| DirEx™ | | 100 | 250-101 | solution |
| DirEx™ *Fast*-Tissue | | 96 T | 260-011 | solution |
| DirEx™ *Fast*-Cultured cell | | 96 T | 260-021 | solution |
| DirEx™ *Fast*-Whole blood | | 96 T | 260-031 | solution |
| DirEx™ *Fast*-Blood stain | | 96 T | 260-041 | solution |
| DirEx™ *Fast*-Hair | | 96 T | 260-051 | solution |
| DirEx™ *Fast*-Buccal swab | | 96 T | 260-061 | solution |
| DirEx™ *Fast*-Cigarette | | 96 T | 260-071 | solution |
| **GeneAll® RNA series** *for preperation of total RNA* | | | | |
| RiboEx™ | mini | 100 | 301-001 | solution |
| | | 200 | 301-002 | |
| Hybrid-R™ | mini | 100 | 305-101 | spin |
| Hybrid-R™ Blood RNA | mini | 50 | 315-150 | spin |
| Hybrid-R™ miRNA | mini | 50 | 325-150 | spin |
| RiboEx™ LS | mini | 100 | 302-001 | solution |
| | | 200 | 302-002 | |
| Riboclear™ | mini | 50 | 303-150 | spin |
| Riboclear™ Plus | mini | 50 | 313-150 | spin |
| Ribospin™ | mini | 50 | 304-150 | spin |
| Ribospin™ II | mini | 50 | 314-150 | spin |
| | | 300 | 314-103 | |
| Ribospin ™ vRD | mini | 50 | 302-150 | spin |
| Ribospin ™ vRD Plus | mini | 50 | 312-150 | spin |
| Ribospin ™ vRD II | mini | 50 | 322-150 | spin |
| Ribospin ™ Plant | mini | 50 | 307-150 | spin |
| Ribospin™ Seed/Fruit | mini | 50 | 317-150 | spin |
| Ribospin™ Pathogen/TNA | mini | 50 | 314-150 | spin |
| | | 250 | 314-152 | |
| Allspin™ | mini | 50 | 306-150 | spin |
| RiboSaver™ | mini | 100 | 351- 001 | solution |

| Products | Scale | Size | Cat. No. | Type |
|---|---|---|---|---|
| **GeneAll® *AmpONE™*** *for PCR amplification* | | | | |
| Taq DNA polymerase | | 250 U | 501-025 | (2.5 U/μl) |
| | | 500 U | 501-050 | |
| | | 1,000 U | 501-100 | |
| Taq Premix | 20 μl x 96 tubes | | 526-200 | solution |
| | 50 μl x 96 tubes | | 526-500 | |
| **GeneAll® *AmpMaster™*** *for PCR amplification* | | | | |
| Taq Master mix | 0.5 ml x 2 tubes | | 541-010 | solution |
| | 0.5 ml x 10 tubes | | 541-050 | solution |
| **GeneAll® *HyperScript™*** *for Reverse Transcription* | | | | |
| Reverse Transcriptase | 10,000 U | | 601-100 | solution |
| RT Master mix | 0.5 ml x 2 tubes | | 601-710 | solution |
| One-step RT-PCR Master mix | 0.5 ml x 2 tubes | | 602-110 | solution |
| One-step RT-PCR Premix | 20 μl x 96 tubes | | 602-102 | solution |
| **GeneAll® RealAmp™** *for qPCR amplification* | | | | |
| SYBR qPCR Master mix (2X, Low ROX) | 200 rxn | 2 ml | 801-020 | solution |
| | 500 rxn | 5 ml | 801-050 | |
| SYBR qPCR Master mix (2X, High ROX) | 200 rxn | 2 ml | 801-021 | solution |
| | 500 rxn | 5 ml | 801-051 | |
| **GeneAll® Protein series** | | | | |
| ProtinEx™ Animal cell/tissue | 100 ml | | 701-001 | solution |
| PAGESTA™ Reducing 5X SDS-PAGE Sample Buffer | 1 ml x 10 tubes | | 751-001 | solution |

| Products | Size | Cat. No. | Type |
|---|---|---|---|
| **GeneAll® GENTi™ 32 ADVANCED** *Newly designed automated extraction system* | | | |
| Automatic extraction equipment | | GTI032A | system |
| Genomic DNA | 48 | 901-048A | tube |
| | 96 | 901-096A | plate |
| Viral DNA/RNA | 48 | 902-048A | tube |
| | 96 | 902-096A | plate |
| Blood DNA | 48 | 903-048A | tube |
| | 96 | 903-096A | plate |
| Plant DNA/RNA | 48 | 904-048A | tube |
| | 96 | 904-096A | plate |
| LMO | 48 | 906-048A | tube |
| | 96 | 906-096A | plate |
| Fecal DNA/RNA | 48 | 913-048A | tube |
| | 96 | 913-096A | plate |
| **GeneAll® AllEx®64** *Compact yet Comprehensive automated extraction system* | | | |
| Automatic extraction equipment | | AEX064 | system |
| Genomic DNA | 48 | 931-048A | tube |
| | 96 | 931-096A | plate |
| Viral DNA/RNA | 48 | 934-048A | tube |
| | 96 | 934-096A | plate |
| Blood DNA | 48 | 935-048A | tube |
| | 96 | 935-096A | plate |
| Plant DNA/RNA | 48 | 937-048A | tube |
| | 96 | 937-096A | plate |
| Fecal DNA/RNA | 48 | 948-048A | tube |
| | 96 | 948-096A | plate |

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2024.06