# DNAmax Animal Genomic DNA Isolation Kit

*For use with animal tissues, adherent cells and cells in suspension*

## *#2401M*

spex sampleprep

# Contents

# Product Description

The DNAmax Animal Genomic DNA Isolation Kit is optimized to work with the Genomax™, Geno/Grinder® and GenoLyte® homogenizers and allows for fast and seamless isolation of DNA from a wide variety of animal tissues and cell types.

**Brief overview**

The DNAmax Animal Genomic DNA Isolation Kits combine the use of chaotropic salts and detergents to lyse animal tissues or cells and release DNA. The addition of Proteinase K and RNase A disassociates the DNA from histones and facilitates the removal of RNA during DNA isolation, respectively. The DNA is captured using a silica column, then washed to remove any contaminants including salts, fats, and proteins. The column is finally dried by centrifugation and DNA is eluted using the elution buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA).

# Included in the kit

| | |
|---|---|
| 1x | ● Lysis buffer A (45 ml) |
| 1x | ● Wash buffer A (30 ml) |
| 1x | ● Elution buffer A (40 ml) |
| 2x | Proteinase K (17 mg / tube) |
| 75x | DNA binding columns and 2.0 ml collection tubes |
| 75x | Homogenization tubes (pre-loaded with 5 stainless steel balls) |
| 2x | RNase A (750 µl / tube) |
| 1x | Protocol |

# Required but not provided

**Equipment**

- Genomax™ or GenoLyte®
- 1.5 ml microcentrifuge tubes
- Microcentrifuge capable of 12,000 x g
- Heat-block capable of 55 °C and shaking at 700 rpm (recommended)
  OR
- Heat-block or water-bath capable of 55 °C
-

**Reagents**

- 1X PBS (Molecular-grade)
- 95 – 100% Ethanol (Molecular-grade)

# Storage

- Proteinase K comes in a powdered form that is stable at room temperature. **Once dissolved, Proteinase K should be stored at -20 °C.** For short-term storage, dissolved Proteinase K can be stored at 4 °C for up to 5 days.
- RNase A should be stored at 4 °C.
- Remainder of kit components may be stored at room temperature.

# Before first use

1. Prepare Proteinase K
   a. Two Proteinase K tubes are provided. Each tube contains enough Proteinase K for approximately 40 reactions. To obtain the working 20 mg/ml solution, **add 850 µl of molecular grade water into each tube**.
   b. Vortex for 1 minute at max speed.
   c. Repeat step b. until no precipitates are present.

   *Proteinase K is ready to use.*

**Storage**

- Once dissolved, Proteinase K can be stored at 2-8 °C for several days.
- For longer storage, aliquot and store Proteinase K at -20 °C. Stable for up to 1 year at -20 °C.
- Proteinase K in lyophilized form is stable at room temperature for up to 1 year.

2. Prepare ● Wash Buffer A
   a. Add **90 ml of 95 - 100% ethanol** into ● **Wash Buffer A**.
   b. Mix by inverting bottle 10 times.

   *Wash Buffer A is ready for use.*

# DNA Isolation Protocol:

# Initial workflow

**Prior to starting initial workflow:**

1. *Ensure Wash Buffer A has been reconstituted with ethanol.*
2. *Pre-heat heat block or water bath to 55 °C.*
3. *If precipitates are present in the lysis, wash or elution buffers, warm at 55 °C and allow precipitates to dissolve. Cool to room temperature before use.*

Starting material: **Animal Tissue**

*This kit has been optimized to work with up to 30 mg of fresh tissue. Starting amounts greater than 30 mg may saturate the DNA binding column, reducing yield and quality of DNA extracted.*
*Depending on the characteristics of tissue used, optimization of the amount of starting material may be required to achieve optimal yield and purity.*

Prepare tissue for homogenization:

1. Measure out 15 – 30 mg of fresh animal tissue directly in the preloaded homogenization tube (Cat #. 2310), containing 5 stainless steel balls (Cat #. 2151).
    a. **Note:** Thaw frozen tissue samples completely. Homogenization of frozen samples will significantly reduce the DNA quality and yield.
2. Add 500 µl of ●**Lysis Buffer A** to each tube.
3. Add **20 µl of 20 mg/ml Proteinase K** to each tube.
4. Ensure caps are tightly secured on tubes.

**If using the Genomax or Geno/Grinder homogenizer:**

a. Insert the tubes in the (Cat #2300) Foam Holder and place in the Genomax platform.

b. Place the lid on the clamp and lower onto the vial. Secure the clamp by turning the knob until finger tight.
   i. **Note**: It is essential to place tubes in all corners of the foam holder to ensure that the clamp is level in the closed position. If less than a full rack of vials is used, be sure to place an equal number of vials on each side of the clamp, to ensure that the load is balanced and tightly clamped. Empty vials can be used as place holders if an uneven number of samples are run.
c. Proceed to homogenization using the following settings on the Genomax:

| | |
|---|---|
| **Cycles** | 1 |
| **Rate** | 1,500 rpm |
| **Run time** | 60 seconds |

*After homogenization, the lysis medium may be heavily foamed. To avoid sample loss or contamination the homogenization tubes can be briefly centrifuged for 10 seconds to collect the liquid at the bottom of the tube.*

d. **Continue to COMMON WORKFLOW (Page 9).**

**<u>If using the GenoLyte homogenizer</u>:**

a. Lift the lid by the handle.
b. Place a vial holder in the clamp. Then add homogenization tubes loaded with up to 30 mg of animal tissue into the holder.
c. Close the arm by pushing it down and then pressing the locking tab inwards until it clasps the clamp arm.
d. Stretch the safety ring around the clamp arm knob to secure the vials in the holder.
e. Close the lid and proceed to homogenization using the following conditions on the GenoLyte:

| | |
|---|---|
| **Cycles** | 1 |
| **Rate** | 4,000 rpm |
| **Run time** | 40 seconds |

*After homogenization, the lysis medium may be heavily foamed. To avoid sample loss or contamination the homogenization tubes can be briefly centrifuged for 10 seconds to collect the liquid at the bottom of the tube.*

f. **Continue to COMMON WORKFLOW (Page 9).**

## Starting material: **Adherent Cell Culture**

1. Remove media and wash cells with 10 ml of 1X **PBS** (room temperature).
2. Aspirate 1X **PBS** and add 500 µl of ●**Lysis Buffer A** directly onto the plate.
3. Scrape cells and pipette into 1.5 ml microcentrifuge tube (not included). Pipette up and down 5 times to achieve homogenous solution.
4. Add **20 µl of 20 mg/ml Proteinase K** to the tube containing cells. Vortex for 10 seconds.
5. ***Continue to COMMON WORKFLOW (Page 9)***

## Starting material: **Cell Suspension**

1. Pellet the cell by centrifuging at 2,000 x g for 3 minutes at room temperature.
2. Remove supernatant and wash the cells by resuspending them gently in 10 ml of 1X PBS.
3. Pellet the cell by centrifuging at 2,000 x g for 3 minutes at room temperature and remove PBS.
4. Resuspend the cell pellet in **500 µl of ●Lysis Buffer A.** Pipette up and down 5 times to achieve a homogenous solution.
5. Add **20 µl of 20 mg/ml Proteinase K** to tube containing cells. Vortex for 10 seconds.
6. ***Continue to COMMON WORKFLOW (Page 9)***

# Common workflow

*Continue here once "Initial workflow" has been completed.*

Proteinase K digestion

1. Incubate homogenization tube at 55 °C for 15 minutes. Recommended: shake at 700 rpm.

Binding DNA to Column

2. Remove tube from heat source.
    a. **Optional Step**: Add 20 µl of RNase A and mix by inversion 5 – 6 times. Let the sample sit for 5 minutes at room temperature.
3. Add **200 µl of 95 – 100% ethanol** to the tube. Vortex at room temperature for 10 seconds.
4. Take up to 700 µl of the solution and pipette into DNA binding column and collection tube (provided).

⚠ **Ensure precipitate or tissue is NOT transferred into column.**

5. Spin the column at 12,000 x g for 1 minute at room temperature.

Washing column

*Note: Ensure **Wash Buffer A** has been previously reconstituted with Ethanol as outline in "Before first use" (Page 5).*

6. Discard flow through and add 700 µl of ●**Wash Buffer A.** Spin column at 12,000 x g for 1 minute.
7. Discard the flow through and add an additional 700 µl of ●**Wash Buffer A** and spin the column at 12,000 x g for an additional 3 minutes

8. Discard the flow through and spin the column for an additional 1 minute at max speed to remove any remaining Buffer A.
9. Remove column and move to fresh 1.5 ml microcentrifuge tube (not included).

Eluting DNA off of column

10. Add 200 µl of the ● **Elution Buffer A** to center of column and incubate at room temperature for 3 minutes.
    *Note: If starting material was low, use less (100 µl) of Elution Buffer A and run through column twice.*

11. Spin the column at max speed for 1 minute and collect the flow through.

**Flow-through contains eluted DNA.**

*High purity DNA will have a 260/280 reading between 1.7 – 2.0 and a 260/230 nm reading of > 2.0. Yields will depend on starting material and can range from approximately 70-250 ng/*µl*.*

Expected yields

| Tissue | Sample Amount | DNA yield (µg) |
|---|---|---|
| Mouse Heart | 30 mg | 9 – 10 |
| Mouse Liver | 30 mg | 20 - 30 |
| Mouse Spleen | 30 mg | 15 – 20 |
| Mouse Kidney | 30 mg | 15 – 20 |
| Beef Loin | 30 mg | 5 – 10 |
| Chicken Breast | 30 mg | 16 – 30 |
| HT1080 (cell line) | 1 x $10^6$ cells | 14 – 30 |
| MEFs (cell line) | 1 x $10^6$ cells | 10 – 12 |
| 1156 (cell line) | 1 x $10^6$ cells | 14 – 30 |

# Troubleshooting

## Sample/wash will not pass through column

**Cause:** Precipitates or tissue fragments are clogging column or starting material was too high and viscous to pass through column using standard spin and time parameters.
**Resolution:** If precipitates or tissue is clogging column, gently pipette the sample in the column to loosen any precipitates and debris and carefully remove them before attempting to pass liquid through again.

If no visible particles are blocking the column, you may increase the time of centrifugation and/or the rpm to force sample or wash through.

Using less starting material or tissue can help prevent column from becoming clogged. When using tissue, ensuring complete homogenization and proper digestion to prevent sample from obstructing and clogging column.

## Low yield of DNA

**Cause:** Ethanol missing from lysate or Wash Buffer A.
**Resolution:** It is critical that ethanol be present in the lysate (step 1) and that the Wash Buffer A be reconstituted with the ethanol prior to first use.

**Cause:** DNA elution was inhibited. Ethanol was not completely removed from column prior to elution step.
**Resolution:** Residual ethanol will inhibit elution of DNA from column. To avoid this, after last wash step, place column in new tube, and spin for at max speed for 3 minutes to ensure all ethanol is removed from column.

**Cause:** Water was used to elute DNA.
**Resolution:** Ensure Elution Buffer A (10 mM Tris-HCl ph 8.0, 1 mM EDTA) is used to elute DNA.

**Cause:** Insufficient homogenization of the tissue.
**Resolution:** If fragments of tissue remain after homogenization, then increase the number of cycles of homogenization to 3 for the Genomax, and 2 for the GenoLyte. It's important to add a REST of 30 seconds between each cycle to avoid heating of the samples during consecutive homogenization cycles.

## 260/280 is outside of expected range (high)

**Cause:** High 260/280 reading may indicate RNA contamination.
**Resolution:** RNase A treatment will remove contamination.

**Cause:** Too much starting material was used. This can cause an overload of the column, and lead to contamination of proteins and low 260/230 and 260/280. A peak at 230 nm indicates contamination of proteins.
**Resolution:** Reduce amount of starting material.

**Cause:** In certain cases, the amount or type of starting material amount may affect downstream wash steps.
**Resolution:** Ensuring tissues are relatively clean from potentially contaminating substances (i.e. using a PBS wash before starting protocol) may result in cleaner DNA.

## 260/280 is outside of expected range (low)

**Cause:** Water was used to elute DNA.
**Resolution:** Ensure Elution Buffer A (10mM Tris-HCl ph8.0, 1mM EDTA) is used to elute DNA.

**Cause:** Silica from column present in elution (very rare).
**Resolution:** Spin down sample at maximum speed for 1 minute. Measure absorbance of supernatant.

## Eluted DNA fails in downstream assay

**Cause:** Salt/Ethanol carried over
**Resolution:** To remove potential contaminating salt or ethanol, add an additional wash step using 700 µl Wash Buffer A and spinning column for 2 minutes at 12,000 x g. After this place the column in a new tube and spin at max speed for 3 minutes to ensure all ethanol is removed from the column.

Additionally, eluting in a larger volume of Elution Buffer will dilute any residual salt or ethanol.

# NOTES

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