



DANAGENE GENOMIC DNA KIT

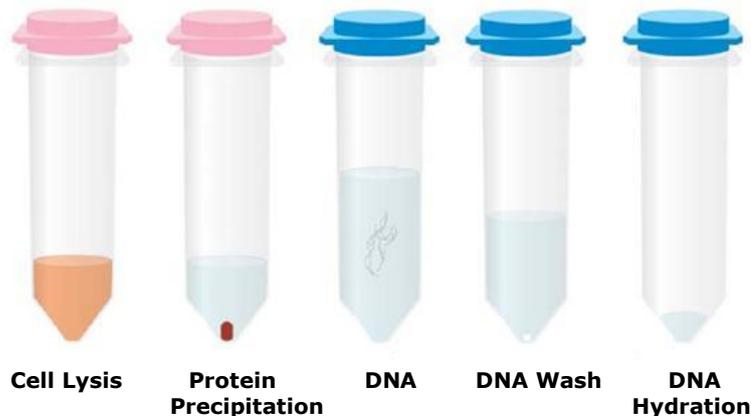
1. INTRODUCTION

1.1 Product Description

This kit is designed for the extraction of high **quality genomic DNA** from a wide variety of samples including cells in culture, animal or human tissues, mouse tails, bacteria and yeast.

For another different samples you can contact with our Technical Service for establish one working protocol.

The process includes a cell lysis with an anionic detergent that solubilizes the necessary cell components, the contaminant RNA can be removed with a RNase treatment. The cell proteins are removed by precipitation, which allows to leave the genomic DNA in solution. Finally, the genomic DNA is isolated by a precipitation with isopropanol.



DNA purified using this kit is highly stable and suited for use in a wide range of applications such as:

- DNA archiving.
- PCR and quantitative real-time PCR.
- SNP analysis.
- Southern Blotting.
- Next Generation Sequencing.

1.2 Kit Components

DANAGENE Genomic DNA Tissue Kit	Ref.0603.1 1 gr o 50 preps 20 mg	Ref.0603.11 4 gr o 200 preps 20 mg	Ref.0603.12 33 gr o 1650 preps 20 mg	T^a Stock
Lysis Solution	32 ml	125 ml	2 x 500 ml	Room T ^a
Protein Precipitation Solution	20 ml	75 ml	2 x 300 ml	Room T ^a
DNA Hydration Solution	15 ml	50 ml	2 x 250 ml	Room T ^a
RNase Proteinase K	175 µl 175 µl	2 x 325 µl 2 x 325 µl	5 ml 5 ml	4°C o – 20°C 4°C o – 20°C

DANAGENE Genomic DNA Cell Kit	Ref.0603.2 50 preps 3-5 x 10⁶	Ref.0603.21 200 preps 3-5 x 10⁶	Ref.0603.22 1650 preps 3-5 x 10⁶	T^a Stock
Lysis Solution	32 ml	125 ml	2 x 500 ml	Room T ^a
Protein Precipitation Solution	20 ml	75 ml	2 x 300 ml	Room T ^a
DNA Hydration Solution	15 ml	50 ml	2 x 250 ml	Room T ^a
RNase	85 µl	325 µl	2.5 ml	4°C o – 20°C

DANAGENE Genomic DNA Mouse Tail Kit	Ref.0603.3 200 preps 0.5-1.0 cm	Ref.0603.31 1650 preps 0.5-1.0 cm	T^a Stock
Lysis Solution	125 ml	2 x 500 ml	Room T ^a
Protein Precipitation Solution	75 ml	2 x 300 ml	Room T ^a
DNA Hydration Solution	50 ml	2 x 250 ml	Room T ^a
Proteinase K	2 x 1000 µl	17 ml	4°C o – 20°C

DANAGENE Genomic DNA Bacteria Kit	Ref.0603.5 200 ml	T^a Stock
Lysis Solution	125 ml	Room T ^a
Lysis Solution Gram +	110 ml	Room T ^a
Protein Precipitation Solution	75 ml	Room T ^a
DNA Hydration Solution	50 ml	Room T ^a
RNase Lysozyme	2 x 325 µl 12 ml	4°C o – 20°C 4°C o – 20°C

DANAGENE Genomic DNA Yeast Kit	Ref.0603.6 200 ml	T^a Stock
Lysis Solution	65 ml	Room T ^a
Lysis Solution Yeast	60 ml	Room T ^a
Protein Precipitation Solution	30 ml	Room T ^a
DNA Hydration Solution	25 ml	Room T ^a
RNase Lyticase	2 x 150 µl 2 x 800 µl	4°C o – 20°C 4°C o – 20°C

1.3 Equipment and reagents necessary and not provided

- Isopropanol.
- 70% Ethanol.
- 1.5 ml and 2.0 ml microtubes, 15 or 50 ml centrifuge tubes.
- Microcentrifuge or clinical centrifuge.
- Vortex.
- Water bath.

1.4 Storage and stability

All components are stable for 12 months from the date of purchase being stored and used as indicated.

2. PROTOCOL

2.1 Preliminary preparations

- If the lysis solution contains a precipitate due to the low temperatures, incubate at 37°C and mix to dissolve the precipitate
- **Store the RNase and the Proteinase K at 4°C. If the using kit period is going to be long, it is recommended to dispense aliquots and to store it at -20°C.**

2.2 General conditions

- It is recommended to include a Proteinase K treatment to increase the lysis efficiency, although this treatment is not necessary with some samples. If you want to reduce the purification time, the user can decide, according to the sample, if he wants the Proteinase K treatment. The addition of 1.5 µl of Proteinase K in 300 µl of Lysis Solution will give a final concentration of 100 µg/ml. Incubate at 55°C for 1 hour or until the digestion is complete.
- If you want DNA free from RNA for the following applications, a digestion with RNase can be done.
- The samples which are difficult to lyse can be pulverised with liquid Nitrogen or can be treated with a mechanical homogenizer.
- Some strains, especially Gram + bacteria and yeast may need a preincubation with lytic enzymes, which are not supplied with this kit.
- If the DNA quantity you hope to obtain is low (< 2 µg), it is recommended to add a carrier such as glycogen (1µl of 20 mg/ml glycogen solution per 600 µl of isopropanol).

2.3 Protocol for genomic DNA from 20 mg / 50 mg of animal tissues

• Cell lysis

1. Dissect the tissue sample and quickly freeze in liquid nitrogen. Store at -70°C or -80°C. It is possible to use fresh tissue directly, work very quickly and keep tissue on ice at all times.
2. Add 20 mg / 50 mg of frozen ground tissue or fresh tissue to 600 µl / 1.5 ml of Lysis Solution. Difficult to treat samples, can be treated with a mechanical homogenizer (Polytron, Ultra turrax).
3. To increase the lysis efficiency in tissues it is recommended to use Proteinase K. Add 3 µl / 7.5 µl mix and incubate at 55°C for 3 hours to overnight night, until the total lysis. If possible, invert tube or shake periodically, with the use of vortex, during the incubation period.

• RNase treatment

1. Add 3 µl / 7.5 µl of RNase to the cell lysate.
2. Mix the sample turning the tube upside down and incubate at 37°C for 15-60 minutes.

• Protein precipitation

1. Cool the sample to room temperature.
2. Add 360 µl / 900 µl of protein Precipitation Solution.
3. Mix with vortex vigorously a high speed for 20-30 seconds.
4. Centrifuge at 14.000 xg / 2.500 xg for 5/10 minutes. The precipitated proteins will form a tight pellet. If the pellet is not visible, repeat step 3 followed by incubation on ice for 5 minutes, then repeat step 4.

• DNA precipitation

1. Pour the supernatant containing the DNA in a 1.5 ml tube containing 600 µl of isopropanol or into a 15 ml tube containing 1.5 ml of isopropanol. Mix by turning the tube upwards several times.
2. Centrifuge at 14.000 xg / 2.500 xg for 3 minutes.
3. Remove the supernatant. Add 600 µl / 1.5 ml of 70% ethanol and invert tube several times to wash the DNA pellet.
4. Centrifuge at 14.000 xg / 2.500 xg for 2 minutes. Remove carefully all the ethanol avoiding touching the pellet.
5. Invert and drain the tube on clean absorbent paper and allow to air dry for 15 minutes.

- **DNA hydration**

1. Add 100 μ l / 250 μ l DNA hydration solution.
2. Incubate at 65°C for 1 hour, shaking to help the DNA hydration, or incubate overnight at room temperature with gentle shaking.
3. Storage at 2-8°C. For long time storage, store at -20°C o -80°C.

Scaled reagents volumes from 5mg up to 600 mg

Tissue quantity in mg	5-10	10-20	25	50	100	300-600
Tube size	1.5 ml	1.5 ml	2.0 ml	15 ml	15 ml	50 ml
Lysis solution	0.3 ml	0.6 ml	0.75 ml	1.5 ml	3.0 ml	18 ml
RNase	1.5 μ l	3.0 μ l	3.75 μ l	7.5 μ l	15 μ l	90 μ l
Proteinase K	1.5 μ l	3.0 μ l	3.75 μ l	7.5 μ l	15 μ l	90 μ l
Protein precipitation sol.	0.18 ml	0.36 ml	0.45 ml	0.90 ml	1.8 ml	10.8 ml
Isopropanol 100%	0.3 ml	0.6 ml	0.75 ml	1.5 ml	3.0 ml	18 ml
DNA Hydration Solution	50 μ l	100 μ l	150 μ l	250 μ l	375 μ l	500 μ l

2.4 Protocol for genomic DNA from 20-30 mg / 40-60 mg of mouse tail

- **Cell lysis**

1. Place 5-10 mm (10-20 mg) / 25 mm (25-50 mg) of mouse tail in 600 μ l / 1.20 ml of lysis solution.
2. Add 10 μ l / 20 μ l of Proteinase K. Mix and incubate at 55°C overnight, or until the total lysis. If possible, mix with vortex during the incubation time.

- **RNase treatment**

1. Add 3 μ l / 6.0 μ l of RNase to the cell lysate.
2. Mix the sample by turning the tube upside down and incubate at 37°C for 15-60 minutes.

- **Protein precipitation**

1. Cool the sample to room temperature.
2. Add 360 μ l / 720 μ l of Protein precipitation solution.
3. Mix with vortex vigorously a high speed for 20-30 seconds.
4. Centrifuge at 14.000 xg / 2.500 xg for 5/10 minutes. The precipitated proteins will form a tight pellet. If the pellet is not visible, repeat step 3 followed by incubation on ice for 5 minutes, then repeat step 4.

- **DNA precipitation**

1. Pour the supernatant containing the DNA in a 1.5 ml tube containing 600 μ l of isopropanol or into a 15 ml tube containing 1.2 ml of isopropanol. Mix by turning the tube upwards several times.
2. Centrifuge at 14.000 xg / 2.500 xg for 3 minutes.
3. Remove the supernatant. Add 600 μ l / 1.2 ml of 70% ethanol and invert tube several times to wash the DNA pellet.
4. Centrifuge at 14.000 xg / 2.500 xg for 2 minutes. Remove carefully all the ethanol avoiding touching the pellet.
5. Invert and drain the tube on clean absorbent paper and allow to air dry for 15 minutes

- **DNA hydration**

1. Add 100-250 μ l / 250-500 μ l DNA hydration solution.
2. Incubate at 65°C for 1 hour, shaking to help the DNA hydration, or incubate overnight at room temperature with gentle shaking.
3. Storage at 2-8°C. For long time storage, store at -20°C o -80°C.

2.5 Protocol for genomic DNA from 1 ml / 5 ml of bacteria cultures

Gram (+) bacteria are more difficult to lysate, for this reason we recommend the incubation with lytic enzymes. For some Staphylococcus species a lysozyme (10 mg/ ml) and lysostaphin (10 mg / ml) mixture is necessary.

• **Cell lysis**

1. Add 1.0 ml / 5.0 ml of an overnight culture to a 1.5 ml / 15 ml tube.
2. Centrifuge at 14.000 xg / 2.500 xg for 30 seconds / 3 minutes. Remove the supernatant. For Gram (+) bacteria continue with step 3. For Gram (-) go directly to step 6.
3. Resuspend the cells in 540 µl / 2.7 ml of Lysis Solution Gram +.
4. Add the correct quantity of lytic enzymes, 60 µl / 300 µl of Lysozyme (10 mg/ml). The purpose of this treatment is to weaken the cell wall to make cell lysis more efficient.
5. Incubate the sample at 37°C for 60 minutes. Centrifuge at 14.000 xg / 2.500 xg for 2 minutes / 5 minutes. Remove the supernatant.
6. Add 600 µl / 3.0 ml of Lysis solution to the cell pellet and mix by pipetting up and down. Incubate the samples at 80°C for 5 minutes. Cool at room temperature.

• **RNase Treatment**

1. Add 3 µl / 15 µl of RNase to the lysate.
2. Mix the sample turning the upside down and incubate at 37°C for 15-60 minutes.

• **Protein precipitation**

1. Cool the sample at room temperature.
2. Add 300 µl / 1500 µl of protein precipitation solution.
3. Mix with vortex vigorously a high speed for 20-30 seconds.
4. Centrifuge at 14.000 xg / 2.500 xg for 5/10 minutes. The precipitated proteins will form a tight pellet. If the pellet is not visible, repeat step 3 followed by incubation on ice for 5 minutes, then repeat step 4.

• **DNA precipitation**

1. Pour the supernatant containing the DNA in a 1.5 ml tube containing 600 µl of isopropanol or into a 15 ml tube containing 3 ml of isopropanol. Mix well.
2. Centrifuge at 14.000 xg / 2.500 xg for 3 minutes.
3. Remove the supernatant. Add 600 µl / 3 ml of 70% ethanol and invert tube several times to wash the DNA pellet.
4. Centrifuge at 14.000 xg / 2.500 xg for 2 minutes. Remove carefully all the ethanol avoiding touching the pellet.
5. Invert and drain the tube on clean absorbent paper and allow to air dry for 15 minutes

• **DNA hydration**

1. Add 200 µl / 500 µl of DNA hydration solution
2. Incubate at 65°C for 1 hour shaking periodically to help the DNA hydration, or incubate overnight at room temperature with gentle shaking.
3. Store at 2-8°C. For long time storage, store at -20°C o -80°C.

2.6 Protocol for genomic DNA from 1 ml / 5 ml of yeast cultures

• **Cell lysis**

1. Add 1.0 ml / 5.0 ml of an overnight culture to a 1.5 ml / 15 ml tube.
2. Centrifuge at 14.000 xg / 2.500 xg for 2 minutes / 5 minutes. Remove the supernatant. Resuspend the cells in 292 µl / 2.7 ml of Lysis Solution Yeast.
3. Add the correct quantity of lytic enzymes, 8 µl / 40 µl of Lyticase (20 mg/ml). This treatment helps the cell lysis.
4. Incubate the sample at 37°C for 60 minutes. Centrifuge at 14.000 x g / 2.500 xg for 2 minutes / 5 minutes. Remove the supernatant.
5. Add 300 µl / 1.5 ml of Lysis solution to the cell pellet and mix by pipetting up and down. Some strains may require at 5 minutes incubation at 65°C.

- **Protein precipitation**

1. Cool the sample at room temperature.
2. Add 150 μl / 750 μl of protein precipitation solution.
3. Mix with vortex vigorously a high speed for 20-30 seconds.
4. Centrifuge at 14.000 $\times\text{g}$ / 2.500 $\times\text{g}$ for 5/10 minutes. The precipitated proteins will form a tight pellet. If the pellet is not visible, repeat step 3 followed by incubation on ice for 5 minutes, then repeat step 4.

- **DNA precipitation**

1. Place the supernatant containing the DNA in a 1.5 ml tube containing 300 μl of isopropanol or into a 15 ml tube containing 1.5 ml of isopropanol. Mix several times.
2. Centrifuge at 14.000 $\times\text{g}$ / 2.500 $\times\text{g}$ for 3 minutes.
3. Remove the supernatant. Add 300 μl / 1.5 ml of 70% ethanol and invert tube several times to wash the DNA pellet.
4. Centrifuge at 14.000 $\times\text{g}$ / 2.500 $\times\text{g}$ for 2 minutes. Remove carefully all the ethanol avoiding touching the pellet.
5. Invert and drain the tube on clean absorbent paper and allow to air dry for 15 minutes

- **DNA hydration**

1. Add 50 μl / 250 μl of DNA hydration solution.
2. Add 1.5 μl / 7.5 μl of RNase.
3. Mix the sample and shake with vortex for 1 second. Centrifuge to collect the sample and incubate at 37°C for 15 minutes.
4. Incubate at 65°C for 1 hour shaking to help the DNA dispersion, or incubate overnight at room temperature with gentle shaking.
5. Store at 2-8°C. For long time storage, store at -20°C or -80°C.

2.7 Protocol for genomic DNA from 3-5 millions / 30-50 million of cell culture

Before the extraction, determine the number of cells using a hemocytometer or other cell counter.

Cells growing in a monolayer can be collected by scraping or by trypsinization. This protocol recommends the scraping method:

- Remove the medium.
- Wash with PBS 1x.
- Collect the cells by scraping with con 2ml of PBS and with the help of a rubber policeman

- **Cell lysis**

1. Add 3-5 million of cells in PBS or culture medium, directly to a 1.5 ml microtube. Or add 30-50 million of cells in PBS or culture medium directly to a 15 ml tube.
2. Centrifuge at 14.000 $\times\text{g}$ / 500 $\times\text{g}$ to precipitate the cells for 10 seconds / 3 minutes. Remove the supernatant, leaving 20-40 μl / 200-400 μl of residual liquid.
3. Vortex vigorously to resuspend the cells in the residual supernatant.
4. Add 600 μl / 6 ml of Lysis Solution and pipet up and down to lyse the cells.
5. Incubate at 55°C for 15 minutes.

- **Treatment with RNase (optional)**

1. Add 1.5 μl / 4.5 μl of RNase to the lysate.
2. Mix the sample by turning the tube upside down and incubate at 37°C for 15-60 minutes.

- **Protein precipitation**

1. Cool the sample at room temperature.
2. Add 360 μl / 3.6 ml of protein precipitation solution.
3. Mix with vortex vigorously a high speed for 20-30 seconds.

4. Centrifuge at 14.000 xg / 2.500 xg for 5/10 minutes. The precipitated proteins will form a tight pellet. If the pellet is not visible, repeat step 3 followed by incubation on ice for 5 minutes, then repeat step 4.

- **DNA precipitation**

1. Pour the supernatant containing the DNA into a 1.5 ml tube with 600 µl of isopropanol or into a tube of 15 ml containing 6 ml of isopropanol. Mix it well by turning the tube upside down several.
2. Centrifuge at 14.000 xg / 2.500 xg for 3 minutes.
3. Remove the supernatant. Add 600 µl / 6 ml of 70% ethanol and and invert tube several times to wash the DNA pellet.
4. Centrifuge at 14.000 xg / 2.500 xg for 2 minutes. Remove carefully all the ethanol avoiding touching the pellet.
5. Invert and drain the tube on clean absorbent paper and allow to air dry for 15 minutes.

- **DNA hydration**

1. Add 100 µl / 500 µl of DNA Hydration Solution.
2. Incubate at 65°C for 1 hour shaking it periodically to help DNA dispersion, or incubate overnight at room temperature with gentle shaking.
3. Storage at 2-8°C. For long storages, store at -20°C o -80°C.

Chart of scaled reagent volume from 1-2 million to 500 millions of cells

Number of cells	1-2 millions	3-5 millions	10 millions	20 millions	30-50 millions	100 millions	500 millions
Tube size	1.5 ml	1.5 ml	15 ml	15 ml	15 ml	50 ml	250 ml
Lysis solution	0.3 ml	0.6 ml	1.5 ml	3.0 ml	6.0 ml	15 ml	75 ml
RNase	1.5 µl	3.0 µl	7.5 µl	15 µl	30 µl	75 µl	375 µl
Protein precipitation sol.	0.18 ml	0.36 ml	0.9 ml	1.8 ml	3.6 ml	9 ml	45 ml
Isopropanol	0.3 ml	0.6 ml	1.5 ml	3.0 ml	6.0 ml	15 ml	75 ml
DNA hydration solution	10-30 µl	30-100 µl	50-150 µl	100-200 µl	200-300 µl	500 µl	2500 µl

3. PROBLEM GUIDE AND POSSIBLE ANSWER

Due to the great sample variety that can be treated to extract genomic DNA, it becomes difficult to generalize possible problems and answers. For this reason, we recommend to contact **DANAGEN- BIOTED** Laboratory Technical Service for any question regarding the protocols or any problem you may have during the process.