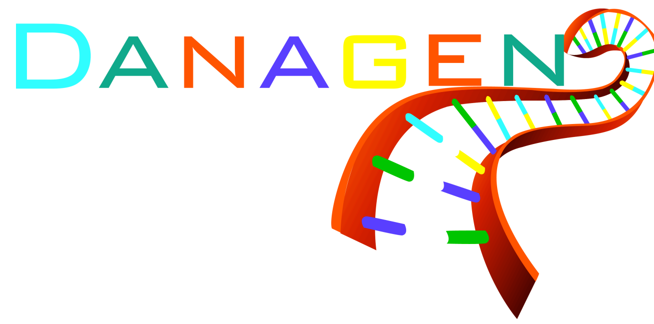

DANAGENE GENOMIC DNA KIT

1.INTRODUCTION

1.1 Prodcut 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	Tª Stock
Lysis Solution	32 ml	125 ml	2 x 500 ml	Room Tª
Protein Precipitation Solution	20 ml	75 ml	2 x 300 ml	Room Tª
DNA Hydratation Solution	15 ml	50 ml	2 x 250 ml	Room Tª
RNase	175 µl	2 x 325 µl	5 ml	4ºC o – 20ºC
Proteinase K	175 µl	2 x 325 µl	5 ml	4ºC o – 20ºC

DANAGENE Genomic DNA Cell Kit	Ref.0603.2 50 preps $3\text{-}5 \times 10^6$	Ref.0603.21 200 preps $3\text{-}5 \times 10^6$	Ref.0603.22 1650 preps $3\text{-}5 \times 10^6$	Tª Stock
Lysis Solution	32 ml	125 ml	2 x 500 ml	Room Tª
Protein Precipitation Solution	20 ml	75 ml	2 x 300 ml	Room Tª
DNA Hydratation Solution	15 ml	50 ml	2 x 250 ml	Room Tª
RNase	85 µl	325 µl	2.5 ml	4ºC o – 20ºC

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	Tª Stock
Lysis Solution	125 ml	2 x 500 ml	Room Tª
Protein Precipitation Solution	75 ml	2 x 300 ml	Room Tª
DNA Hydratation Solution	50 ml	2 x 250 ml	Room Tª
Proteinase K	2 x 1000 µl	17 ml	4ºC o – 20ºC

DANAGENE Genomic DNA Bacteria Kit	Ref.0603.5 200 ml	Tª Stock
Lysis Solution	125 ml	Room Tª
Lysis Solution Gram +	110 ml	Room Tª
Protein Precipitation Solution	75 ml	Room Tª
DNA Hydratation Solution	50 ml	Room Tª
RNAse	2 x 325 µl	4ºC o – 20ºC
Lysozime	12 ml	4ºC o – 20ºC

DANAGENE Genomic DNA Yeast Kit	Ref.0603.6 200 ml	Tª Stock
Lysis Solution	65 ml	Room Tª
Lysis Solution Yeast	60 ml	Room Tª
Protein Precipitation Solution	30 ml	Room Tª
DNA Hydratation Solution	25 ml	Room Tª
RNAse	2 x 150 µl	4ºC o – 20ºC
Lyticase	2 x 800 µl	4ºC o – 20ºC

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 37ºC and mix to dissolve the precipitate
- **Store the RNase and the Proteinase K at 4ºC. If the using kit period is going to be long, it is recommended to dispense aliquots and to store it at –20ºC.**

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 mg/ml. Incubate at 55ºC 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 homogenizator.
- 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 mg), 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 –70ºC or –80ºC. 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 homogenizator (Polytron, Ultra turrax).
3. To increase the lysis efficiency in tissues it is recommended to use Proteinase K. Add 3 ml / 7.5 ml mix and incubate at 55ºC 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 37ºC 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 hydratation**

1. Add 100 µl / 250 µl DNA hydratation solution.
2. Incubate at 65ºC for 1 hour, shaking to help the DNA dispersion, or incubate overnight at room temperature.
3. Storage at 2-8ºC. For long time storage, store at −20ºC o −80ºC.

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 µl	3.0 µl	3.75 µl	7.5 µl	15 µl	90 µl
Proteinase K	1.5 µl	3.0 µl	3.75 µl	7.5 µl	15 µl	90 µl
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
Hydratation Solution	50 µl	100 µl	150 µl	250 µl	375 µl	500 µl

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 µl / 1.20 ml of lysis solution.
2. Add 10 µl / 20 µ l of Proteinase K. Mix and incubate at 55ºC overnight, or until the total lysis. If possible, mix with vortex during the incubation time.

- **RNase treatment**

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

- **Protein precipitation**

1. Cool the sample to room temperature.
2. Add 360 µl / 720 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 1.2 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 µl / 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 hydratation**

1. Add 100-250 µl/ 250-500 µl DNA hydratation solution.
2. Incubate at 65ºC for 1 hour, shaking to help the DNA dispersion, or incubate overnight at room temperature.
3. Storage at 2-8ºC. For long time storage, store at −20ºC o −80ºC.

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 Lysozime (10 mg/ ml) and lysostafine (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). This process makes easier the cell lysis.
5. Incubate the sample at 37ºC 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 use the pipette for resuspending and lysate the cells. Incubate the samples at 80ºC for 5 minutes. Cool at room temperature.

- **RNase Treatment**

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

- **Protein precipitation**

1. Cool the sample at room temperature.
2. Add 300 ml / 1500 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 in a 1.5 ml tube containing 600 ml 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 ml / 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 hydratation**

1. Add 200 µl / 500 µl of DNA hydratation solution
2. Incubate at 65ºC for 1 hour shaking periodically to help the DNA dispersion , or incubate overnight at room temperature for it to rehydrate.
3. Store at 2-8ºC. For long time storage , store at –20ºC o –80ºC.

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 37ºC 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 use the pipette for resuspending and lyse the cells. Some strains may require at 5 minutes incubation at 65ºC.

- **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 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. Place the supernatant containing the DNA in a 1.5 ml tube containing 300 ml of isopropanol or into a 15 ml tube containing 1.5 ml of isopropanol. Mix several times.
2. Centrifuge at 14.000 xg / 2.500 x 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 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 hydratation**

1. Add 50 µl / 250 µl of DNA hydratation 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 37ºC for15 minutes.
4. Incubate at 65ºC for 1 hour shaking to help the DNA dispersion, or incubate overnight at room temperature for it to rehydrate.
5. Store at 2-8ºC. For long time storage, store at –20ºC o –80ºC.

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

Before the extraction, determine the number of cell using a hemocitometer or other cell counter.
Cells growing in a monolayer can be collected by scraping or by tripsinitation. 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 millions of cells in PBS or culture medium, directly to a 1.5 ml microtube. Or add 30-50 millions of cells in PBS or culture medium directly to a 15 ml tube.
2. Centrifuge at 14.000 xg / 500 xg 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 600µl / 6 ml of Lysis Solution and pipet up and down to lyse the cells.
5. Incubate at 55ºC for 15 minutes.

- **Treatment with RNase (optional)**

1. Add 1.5 µl / 4.5 µl of RNase to the lysate.
2. Mix the simple by turning the tube upside down and incubate at 37ºC 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 hydratation**

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

Chart of scaled reagent volume from 1-2 millions 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 hydratation sol.	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.