TREVIGEN® Instructions

For Research Use Only. Not For Use In Diagnostic Procedures

Genomic DNA Isolation Kit

Cat #: 4850-20-GD

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I. Introduction

Trevigen's Genomic DNA Isolation Kit is designed to overcome the most common problems associated with genomic DNA isolation, including endogenous nucleases and shearing. The kits employ a non-phenol:chloroform-based, biphasic extraction to prepare highly purified genomic DNA preparations that are free of protein and RNA. No columns or multiple organic extractions are required. DNA isolated from this kit is suitable for PCR, DNA hybridization, array-based experiments, and restriction enzymes digestions.

II. Precautions and Limitations

1. For Research Use Only. Not for use in diagnostic procedures.
2. The physical, chemical, and toxicological properties of the products contained in the Genomic DNA Isolation Kit may not yet have been fully investigated. Therefore, Trevigen recommends the use of gloves, lab coats, and eye protection while using any of these chemical reagents. Trevigen assumes no liability for damage resulting from handling or contact with these products.

III. Materials Supplied

Component	Amount Provided	Storage	Cat#
Lysis Solution 1	2 x 1 ml	Room temp.	4850-20-01
Extraction Solution 2	20 ml	Room temp.	4850-20-02
Extraction Buffer 3	8 ml	Room temp.	4850-20-03
Sodium Acetate 4	1 ml	Room temp.	4850-20-04
DNase-free Water 5	2 ml	Room temp.	4850-20-05
Sample Buffer 6	2 ml	Room temp.	4850-20-06

IV. Materials/Equipment Required but Not Supplied

Equipment:

1. 1-20 µl pipettor
2. 20-200 µl pipettor
3. 200-1000 µl pipettor
4. microcentrifuge
5. vortex
6. UV spectrophotometer
7. -20 °C and 4 °C storage

Reagents:

1. 2-propanol (isopropanol)
2. 70% ethanol

Disposables:

1. 1-200 µl pipette tips
2. 100-1000 µl pipette tips
3. 1.5 ml Microcentrifuge tubes
4. Gloves (e.g. latex)

V. Assay Protocol

A. DNA Isolation from Tissues:

For DNA extraction from tissues, purchase Tissue Supplement Reagents (*Cat# 4859-20-K) separately.*

Note: For best results, process the tissue sample immediately after harvesting. It is recommended that you proceed with the entire protocol without stopping. If necessary, samples may be frozen at -20 or -80 °C after steps 3 or 9 of the DNA Isolation procedure. If processing cells from culture, proceed to DNA Isolation from Cultured Cells.

1. Mince the tissue into smaller pieces and freeze in liquid nitrogen.

2. Grind the frozen tissue samples into powder using either a pre-chilled hammer or mortar and pestle to avoid thawing of the sample. Add additional liquid nitrogen if required. Process 0.2-1 gram of the powder at one time. Please consider the amount of material being processed as only 20 x 100 µl samples can be processed in the DNA Laddering Kit (if used).

3. Suspend the tissue into Sample Buffer in the following ratio:

   0.1 g of powdered tissue sample
   200 µl of Sample Buffer (Cat# 4850-20-06)
   20 µl of 10X Tissue Buffer (Cat# 4859-20-01)

   Incubate at 50 °C for 12 to 18 hours. Gentle shaking is recommended.

4. Transfer 100 µl of the cell suspension from step 3 to a new microcentrifuge tube. Add 100 µl Lysis Solution 1 (Cat# 4850-20-01) and mix thoroughly by inverting tube several times.

   Note: The remaining cell suspension may be frozen at -80°C for future use.

   Continue with step 4 of part V.B. - DNA Isolation from Cultured Cells.

B. DNA Isolation from Cultured Cells:

Note: For best results, process samples immediately after harvesting.

1. **Suspension Cells:** Harvest cells (10^5 to 10^7) by centrifugation and resuspend the pellet in 100 µl of Sample Buffer (10^6 to 10^8 cells/ml). Proceed to step 3.

2. **Adherent Cells:** Lyse cells directly in culture dish by adding 100 µl of Sample Buffer and 100 µl of Lysis Solution 1. Gently scrape cells and pipet until completely in solution. Proceed to step 5.

3. Incubate this cell suspension at room temperature for 5 to 15 minutes.

4. Lyse the sample and stabilize the DNA by adding 100 µl of Lysis Solution 1. Mix thoroughly by inverting tube several times.

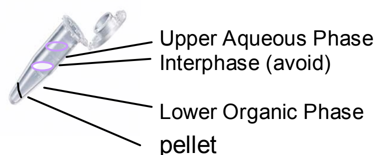
5. Make sure that the sample is now in a 1.5-2 ml microcentrifuge tube. Shake Extraction Solution 2 and then add 700 µl to the sample.

6. Add 400 µl of Extraction Buffer 3 to the sample. Vortex for 10 seconds.

7. Centrifuge at 12,000 x g for 5 minutes.

8. Transfer the upper layer (aqueous) to a new microcentrifuge tube. Avoid removing the darker, lower organic and interphase layer. If the recovered aqueous layer is cloudy, repeat steps 7 and 8. Discard the organic layer by protocols established in your laboratory.

Note: If the aqueous layer is very cloudy, a chloroform extraction may be required. This can be performed by adding 400 µl of chloroform to the aqueous layer in a fume hood. Vortex the sample for 10 seconds, and repeat steps 7 and 8.



When removing the upper phase:
Carefully remove the upper aqueous phase without disturbing the interphase.

If the interphase is accidentally removed, centrifuge the tube again and carefully remove the upper layer.

9. Add 40 µl (*i.e.* 0.1 volume) of Sodium Acetate 4 to the aqueous sample that was transferred. Mix by inverting.
10. Add 440 µl (*i.e.* equal volume) of 2-propanol and mix by inverting.
    Note: Optional stopping point by placing samples at -20 °C overnight.
11. Centrifuge at 12,000 x g for 10 minutes at room temperature.
12. Carefully remove the supernatant without disturbing the pellet. Add 1 ml of 70% ethanol to the pellet. Mix gently by inverting the tube several times.
13. Centrifuge at 12,000 x g for 5 minutes at room temperature.
14. Carefully remove the supernatant without disturbing the pellet. Allow the pellet to dry by inverting the tube on a laboratory tissue and allowing the liquid to evaporate, or with the aid of a vacuum centrifuge apparatus.
15. Resuspend the pellet in 100 µl of DNase-free Water 5.
16. Quantitate the DNA spectrophotometrically. Take 5 µl of the DNA sample and add to 1 ml of water. Determine the optical density at 260 nm against a water blank. The DNA concentration of the undiluted sample can be determined as:

    concentration (µg/µl) = Absorbance_{260} of diluted sample x 9.88*

* The 9.88 is a multiplication factor that accounts for the 1:200 dilution and the O.D. of the DNA in H_2O.

Alternatively, the DNA concentration can be estimated from an ethidium bromide stained gel by comparing with a sample of DNA of known concentration.

VI. Troubleshooting Guide

PROBLEM	POSSIBLE CAUSE	RECOMMENDATIONS
Precipitate in Lysis Solution	Solution was chilled	Warm to 37°C and mix by inversion until all precipitates are dissolved
DNA Recovery	Inadequate lysis of sample	Mix equal volumes of Lysis Solution 1 with sample in Sample Buffer
Phase Inversion	Lysis Solution 1 not diluted sufficiently	Make sure to add equal volumes of Lysis Solution 1 with the sample in Sample Buffer.
	Lysate too viscous	Resuspend the sample in a larger volume of Sample Buffer and Lysis Solution 1.

VII. Reference

1. Rosl, F. 1992. A simple method for detection of apoptosis in human cells. *Nucleic Acid Research* **201**:5243.

VIII. Related Products

Catalog #	Description	Size
9610-5-D	**Salmon Sperm DNA**	**5 x 1 ml**
9605-5-D	**Herring Sperm DNA**	**5 x 1 ml**
9600-5-D	**Calf Thymus DNA**	**5 x 1 ml**
4850-20-ET	**TACS® Apoptotic DNA Laddering Kit**	**20 Samples**

The product accompanying this document is intended for research use only and is not intended for diagnostic purposes or for use in humans.

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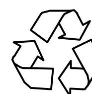

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