Tissue and cells genomicPrep Mini Spin Kit

GENOMIC DNA PURIFICATION

Tissue and cells genomicPrep Mini Spin Kit (Fig 1) is designed for the rapid extraction and purification of high molecular weight genomic DNA (gDNA) from a variety of animal tissues and mammalian cell cultures. Extraction and purification takes just 90 minutes for tissues and 45 minutes for cells, from sample to elution. DNA produced by the kit supports molecular biology applications including cloning, restriction enzyme digestion, genotyping, end-point and real-time PCR, and next generation sequencing (NGS).

Tissue and cells genomicPrep Mini Spin Kit delivers:

- **Fast results:** From tissue sample to high-quality gDNA in just 90 minutes.
- **Simpler purification:** Color-coded caps and bottles with matching protocol steps minimize the chance for error; quick reference protocol card provides instructions at a glance for experienced users.
- **High quality and purity:** Optimized tissue protocol produces intact, RNA-free gDNA that is > 20 kb in size (Fig 2) with a purity of 1.8 (A_{260}/A_{280}).
- **High yields:** Consistently high yields from animal tissue.

Method overview

We have optimized the Tissue and cells genomicPrep Mini Spin Kit to deliver speed with quality. Isolation of gDNA from animal tissues can be completed in about 90 min, and the protocols have been designed to minimize shearing, resulting in high-quality, intact gDNA. The procedure uses a lysis solution in combination with proteinase K and chaotropic agents to release gDNA from various tissue samples and mammalian cells, denature protein components, and promote the selective binding of DNA to a silica-membrane column (1). Contaminants are removed in subsequent wash steps and gDNA is eluted with prewarmed buffer. Buffer chemistry and protocols have been designed to accommodate gDNA extraction from animal tissue and mammalian cell samples.

Fig 1. Tissue and cells genomicPrep Mini Spin Kit for rapid extraction and purification of genomic DNA from animal and mammalian cell cultures.

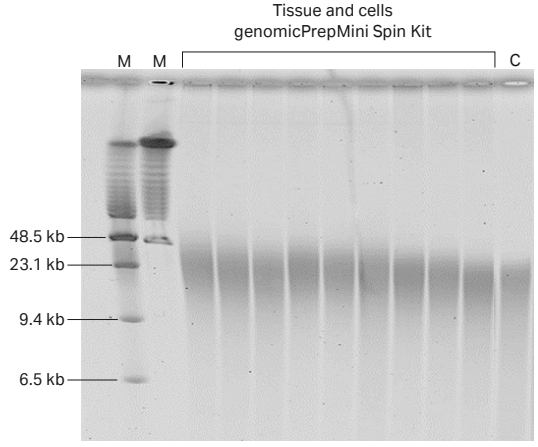

Fig 2. Sizing by pulsed-field gel elctrophoresis (PFGE) of gDNA purified from rat liver. Genomic DNA was purified according to standard instructions. Samples contained 200 ng purified gDNA. C = control rat gDNA. M = molecular weight markers.

You can use this kit to extract gDNA from a variety of tissue types, such as liver, kidney, and mouse tails, and mammalian cell lines, including CHO and human lung fibroblasts. Specifications are shown in Table 1.

Table 1. Specifications for Tissue and cells genomicPrep Mini Spin Kit

Feature	Specification	
Sample type	Animal tissue	Cultured cells
Sample input size	5 to 50 mg of animal tissue	Up to 5 × 106 cultured cells
Elution volume	200 µL	200 µL
Number of steps	5	5
Binding capacity	> 35 µg	> 35 µg
Typical yield	0.5–1.5 µg DNA/mg of animal tissue*	10 to 20 µg of gDNA (from 5 × 10^6 cells)
Purity (A_{260}/A_{280})	> 1.75	> 1.75
Time per prep	90 min	45 min
Product size	> 20 kb	> 20 kb

* Values shown derived from rat liver samples; actual yields will vary depending on tissue type used.

High quality and purity

The Tissue and cells genomicPrep Mini Spin Kit yields high quality gDNA with sizes larger than 20 kb. The purified gDNA obtained from rat liver tissue with the Tissue and cells genomicPrep Mini Spin Kit was of high molecular weight with minimal shearing (Fig 2). The purity of the Tissue and cells genomicPrep DNA was also high (Tables 2 and 3). A purity ratio of 1.7 to 1.9 indicates that the gDNA is pure for all standard molecular biology applications.

The Tissue and cells genomicPrep Mini Spin Kit also yields intact, well-sized gDNA from cultured mammalian cells. Figure 3 shows pulsed-field gel electrophoresis results for gDNA obtained from human MRC5 cells, which reveals that gDNA was highly intact at nearly 75 kb.

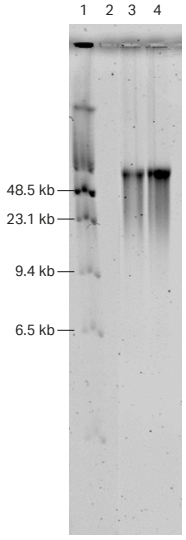

Fig 3. Pulsed-field gel electrophoresis of gDNA purified from MRC5 cells using the Tissue and cells genomicPrep Mini Spin Kit. Two separate preparations from different operators are shown (lanes 3 and 4). Lane 1 contains a low-range PFGE marker. Lane 2 was empty.

Consistently high yields

The capacity of the Tissue and cells genomicPrep Mini Spin columns is > 35 µg and the columns can handle up to 50 mg of animal tissue. These two factors, together with an optimized protocol, result in high yields of gDNA (Table 2).

Table 2. Yield performance of Tissue and cells genomicPrep Mini Spin Kit*

Kit	Yield (µg) ± sd	Purity (A_{260}/A_{280}) ± sd
Tissue and cells genomicPrep Mini Spin Kit	21.2 ± 4	1.84 ± 0.01

* Testing was performed using 20 mg of rat liver tissue. Six different rat livers were used for DNA purification. The data is an average of 54 genomicPrep Mini Spin preparations using three different operators.

Table 3 summarizes yields obtained from a variety of animal tissues and cell lines using the Tissue and cells genomicPrep Mini Spin Kit. High gDNA yields are obtained from tissue types such as kidney and mouse tails. Genomic DNA isolation results from cultured lung cells demonstrate the high capacity of the silica resin employed by the kit. Inputs ranging from 1 to 5 million cells give sufficient gDNA for downstream applications.

Table 3. gDNA yield from varying tissues, cells, and input amounts

Tissue type	Sample amount	Yield (µg) ± sd	Purity (A_{260}/A_{280}) ± sd
Rat liver (n = 54)	20 mg	21.17 ± 4	1.84
Rat kidney (n = 4)	15 mg	19.56 ± 1.7	1.81
Mouse tail (n = 6)	15 mg	11.7 ± 1.7	1.84
CHO cells	1 × 10^5 cells	1.52	1.97
	3 × 10^5 cells	2.57	1.88
	5 × 10^5 cells	3.62	1.88
	1 × 10^6 cells	5.22	1.86
	3 × 10^6 cells	8.79	1.85
	5 × 10^6 cells	13.89	1.84
Kidney HK293 cells (n = 2)	5 × 10^5 cells	16.95 ± 7.3	1.88
Lung MRC5 cells (n = 2)	5 × 10^5 cells	48.36 ± 6.7	1.86

Compatibility with downstream applications

Restriction enzyme digestion

The purity and concentration of gDNA isolated from mammalian tissues using Tissue and cells genomicPrep Mini Spin Kit enables its direct use in restriction enzyme digestions. Tests with several enzymes, including HindIII, EcoRI, and BamHI, demonstrated that purified gDNA from animal tissues was free from restriction enzyme inhibitors. Results for HindIII with gDNA isolated from rat liver tissue are shown in Figure 4.

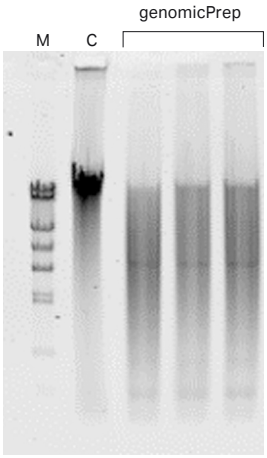

Fig 4. HindIII digest of gDNA. Purified gDNA from the Tissue and cells genomicPrep Mini Spin Kit was cut with HindIII. M = Lambda-HindIII molecular weight marker; C = uncut gDNA purified with Tissue and cells genomicPrep Mini Spin Kit.

Genomic DNA isolated from cultured cells is also suitable for use in restriction enzyme digestions. gDNA isolated from CHO cells using the Tissue and cells genomicPrep Mini Spin Kit was readily digested, without inhibition of HindIII restriction enzyme activity (Fig 5). The inability to remove RNA (despite RNase treatment) can result in restriction digest failures. RNase A treatment with the Tissue and cells genomicPrep Mini Spin Kit leaves purified DNA free of such inhibitors that may copurify with RNA.

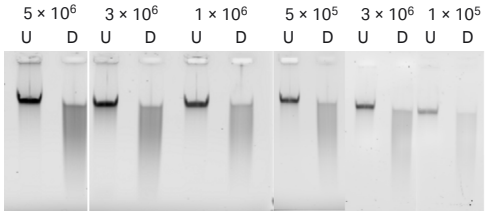

Fig 5. HindIII restriction enzyme digest of gDNA purified from CHO cells using the Tissue and cells genomicPrep Mini Spin Kit. Each digestion reaction contained 20 µL gDNA, and digests were analyzed on 0.8% agarose gel. U = uncut DNA (no enzyme); D = gDNA digested with HindIII. Numbers represent cell amounts from which gDNA was isolated.

Real-time PCR

DNA purified using the Tissue and cells genomicPrep Mini Spin Kit performs effectively in quantitative real-time PCR. The sensitivity of gDNA purified using the Tissue and cells genomicPrep Mini Spin Kit was investigated by performing quantitative real-time PCR analysis. The gDNA isolated from rat liver tissue performed equivalently to the standard rat gDNA control, indicating that the DNA was free from PCR inhibitors (Fig 6).

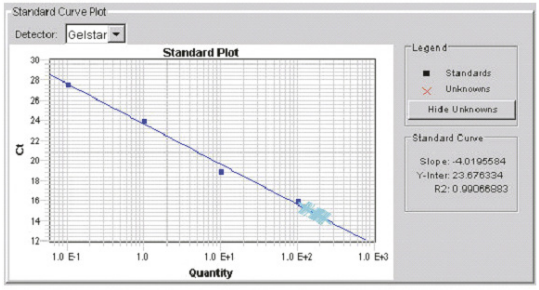

Fig 6. Real-time PCR analysis of gDNA purified from rat liver tissue. Amplification of rat GAPDH gene was performed using 100 ng gDNA purified using the Tissue and cells genomicPrep Mini Spin Kit (n=12). The standard curve was generated with 0.1-100 ng of commercially available rat gDNA (Bioline). The average Ct value for the Tissue and cells genomicPrep Mini Spin Kit was 14.64 ± 0.38.

Similar results were found following qPCR on gDNA purified from HK293 cell lines (Fig 7).

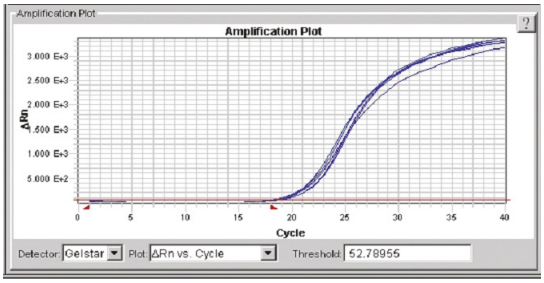

Fig 7. Real-time PCR of gDNA purified from HK293 cells. Amplification reactions used 100 ng gDNA produced by the Tissue and cells genomicPrep Mini Spin Kit

Summary

The Tissue and cells genomicPrep Mini Spin Kit rapidly isolates gDNA from a variety of animal tissues and mammalian cell cultures with a total time to extract gDNA of less than 90 minutes. Genomic DNA purifications performed with the Tissue and cells genomicPrep Mini Spin Kit generate consistent yields with high molecular weight across different sample types. The intact and highly pure gDNA can be used directly in restriction digestion or real-time PCR.

References

1. Vogelstein, B. and Gillespie, D. Preparative and analytical purification of DNA from agarose, *Proc. Natl. Acad. Sci. USA* **76**, 615–619 (1979).

Ordering information

Product	Quantity	Product code
Tissue and cells genomicPrep Mini Spin Kit	50 preps	28904275
	250 preps	28904276

cytiva.com/genomicPrep

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