Spin Doctor Genomic DNA Isolation Kit PROTOCOL

Description: The Spin Doctor Genomic DNA Isolation Kit provides for rapid and consistent isolation of genomic DNA from rodent tail and ear punch samples. DNA is ready for any application including PCR and Southern Blotting.

Important: This document contains two different versions of the protocol. Please use the one that best suits your downstream application.

For PCR: Please use the rapid 15 minute version located on page 2.

For Southern Blotting: Please use the 30 minute protocol starting on page 3.

For Use With Cultured Cells: Please review the additional notes on page 5.

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SPIN DOCTOR GENOMIC DNA PREP KIT
RAPID PROTOCOL

Rapid version suitable for PCR with robust primers (15MIN.)

Important
The rapid version of the protocol produces DNA suitable for PCR analysis with most primers, taking 15 minutes after sample digestion. It is not recommended for Southern Blot analysis or PCR with less robust primers - in this case please use the standard protocol.

A 2-3mm mouse tail sample or 1-2mm rat tail sample will generate sufficient DNA for most applications, and the digest will be complete in approximately 2 hours. Larger tail sections can be used (up to 1.2cm for mouse or .5cm for rat) but require longer digestion times. Larger sample sizes can also be used to obtain higher yields and concentrations.

Sample size will dictate lyses/digestion time – please refer to incubation chart

The largest sample size that can be used is 1.2cm from a mouse and .5cm from a rat – these sample sizes will require longer digestion times

Protocol

1. Add tail or ear punch sample to the Genomic Isolation Tube
2. Resuspend the Genomic Isolation Tube using 500 uL of Genomic Resuspension Buffer and Incubate at 57° C for 1 – 3 hours
   A. Mix by vortexing briefly
   B. Periodic vortexing will help speed digestion
   C. The digest is complete once the tissue has been dissolved (bone and hair might remain) – please refer to incubation chart for approximate digestion times

Incubation Chart	
Sample Size	**Digestion Time**
2mm	Under 2 hours
3mm	2.5 hours
6mm	3 hours
1.2cm	Overnight

* Tubes can be left to digest overnight if more convenient

3. Add 800 uL of Genomic DNA Wash Buffer and mix by inverting 30 times or by vortexing
4. Add 600 uL of Isopropanol and mix by inverting 30 times or by vortexing
5. Spin at 14,000 g for 5 minutes
   A. Decant supernatant by pouring it off and then pipetting off remains
   B. Allow samples to air dry for 2 minutes
6. Resuspend in 250 uL of Final Resuspension Buffer (10mM Tris pH 8.0)
   A. Vortex to re-suspend DNA
7. Spin at 14,000-16,000 g for 5 minutes
   A. Transfer 200 uL of the supernatant into a clean tube, use care not to transfer any hair or solids
   B. DNA is ready for PCR

Tail hair is NOT a problem – hair will pellet down with DNA

The supernatant contains clean genomic DNA – hair and solids can be discarded

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SPIN DOCTOR GENOMIC DNA PREP KIT
STANDARD PROTOCOL

Standard version suitable for Southern Blot anaylsis or PCR with less robust primers (30 MIN.)

Important
The standard version is recommended for Southern Blot analysis or PCR with less robust primers. The protocol takes approximately 30 minutes after samples have been digested.

A 2-3mm mouse tail sample or 1-2mm rat tail sample will generate sufficient DNA for most applications, and the digest will be complete in approximately 2 hours. Larger tail sections can be used (up to 1.2cm for mouse or .5cm for rat) but require longer digestion times. **Recommended :** Larger sample should be used to obtain higher yields and concentrations for Southern Blot analysis.

Protocol

1. Add tail or ear punch sample to the Genomic Isolation Tube
   - The tubes contain a lyophilized blend of enzymes developed to rapidly and effectively lyse cells

Sample size will dictate lyses/digestion time – please refer to incubation chart

2. Resuspend the Genomic Isolation Tube using 500 uL of Genomic Resuspension Buffer
   A. Mix by inverting 10-15 times

The largest sample size that can be used is 1.2cm from a mouse and .5cm from a rat – these sample sizes will require longer digestion times

3. Incubate at 57° C for 1-3 hours – refer to chart below
   A. Inverting periodically will help speed up digestion time
   B. The digest is complete once the tissue has been dissolved (bone and hair might remain) – please refer to incubation chart for approximate digestion times

Some Southern Blots require higher concentrations and yields – Please use larger sample sizes if this is the case

Incubation Chart	
Sample Size	**Digestion Time**
2mm	**Under 2 hours**
3mm	**2.5 hours**
6mm	**3 hours**
1.2cm	**Overnight**

* Tubes can be left to digest overnight if more convenient

-Please Continue-

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4. Add 800 uL of Genomic DNA Wash Buffer
   A. Mix by inverting 30 times
      - The wash buffer will retain the contaminating proteins, nucleases, and degraded nucleic acids

Tail hair is NOT a problem – hair will pellet down with DNA

5. Add 600 uL of Isopropanol
   A. Mix by inverting 30 times
      - The Isopropanol will precipitate genomic DNA while contaminants will be retained in the wash solution

6. Spin at 14,000 g for 10 minutes
   A. Decant supernatant by pouring it off
      - Pelleting the DNA will allow you to remove the contaminants by simply discarding the supernatant

7. 70% Ethanol wash
   A. Add 1 mL of 70% Ethanol to each tube
   B. Mix by inverting 20 times
   C. Microcentrifuge at maximum speed for 2 minutes
   D. Discard supernatant by pouring it off
   E. Repeat steps 7.a-7.d once
      - Ethanol wash steps eliminate contaminating salts

8. Pipette off remaining Ethanol, and air dry for 10 minutes
      - Air drying removes contaminating Ethanol

If wide bore pipette tips are not available improvise by cutting off the very end of a standard tip

9. Resuspend in 250 uL of Final Resuspension Buffer (10mM Tris pH 8.0)
   A. Incubate at 57°C for 10 minutes
   B. Pipette midway through the incubation to help loosen the DNA pellet, and once more after incubation. Use wide bore tips in order to prevent shearing of the DNA

The supernatant contains clean genomic DNA – hair and solids can be discarded

10. Spin at 14,000-16,000 g for 5 minutes
    A. Transfer 200uL of the supernatant into a clean tube, use care not to transfer any hair or solids
    B. DNA is ready for any downstream application
       - This final spin is crucial because it removes any DNA which has not re-suspended as well as any remaining solids

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NOTES FOR ISOLATING DNA FROM CULTURED CELLS

Protocol Selection
Either of the included protocols may be used to isolate DNA from cultured cells with few modifications. Similar to working with other types of samples, the rapid protocol is recommended for PCR while the extended protocol is best for Southern Blots.

Starting Sample Size
Final yield will vary dependant upon the starting sample size and specific cell line. For best results, start with 200,000 to 400,000 cells. This will yield minimum concentrations of 200ng/uL when eluted into 100uL of the Final Re-Suspension Buffer, and total yield will normally be above 200ug from this sample size. Larger starting samples can be used, but could result in extremely high concentrations of DNA when the prep is complete and these may be difficult to re-suspend.

Cell Preparation
Before beginning the kit protocol the cells should be Trypsinized and washed with PBS. Spin down the cells and re-suspend them using the included **Genomic Resuspension Buffer.**

Digestion Times
Cultured cells will digest very rapidly in Proteinase K. Digesting for 1 hour will guarantee a complete digest when working with 200,000 to 400,000 cells and in many instances a shorter digest may be used with successful results.

Final Re-Suspension
After completing the protocol, for best results re-suspend in 100uL of **Final Resuspension Buffer** in order to increase the concentration of the DNA. Larger volumes of **Final Resuspension Buffer** may be used, up to 250uL depending upon individual needs.

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