Sidewinder™ Genomic DNA Purification Kit

Sidewinder Genomic DNA Purification Kit

Sidewinder Genomic DNA Purification Kit 30 rxn 70686-3

Description

The Sidewinder™ Genomic DNA Purification Kit provides simple, small-scale isolation of high quality genomic DNA from a wide range of animal tissues. Based on a modified salting-out procedure, the kit does not employ a chromatographic step or organic extractions. The kit uniquely includes disposable homogenization devices, making the entire procedure very convenient and reproducible.

The kit is designed to perform 30 isolations, each using from 2 mg to 50 mg of fresh or frozen tissue. Typical yields range from 0.5 to 3 µg DNA per mg of tissue, depending on the source. The majority of DNA isolated is about 40 kbp in size and is fully compatible with all applications involving restriction digestion and PCR amplification.

Components

- 13.5 ml Solution 1
- 1.4 ml Solution 2
- 11 mg Lyophilized Solution 3
- 10 ml Solution 4
- 1 ml Sterile Water
- 6 ml TE Buffer
- pkg/30 Homogenization Tubes plus Resin
- pkg/30 Pestles
- pkg/33 Spin Filters
- pkg/33 Large Bore Pipet tips

Storage

Store reconstituted Solution 3 at –20°C. All other components can be stored at room temperature.

Average genomic DNA yields from tissues using the Sidewinder kit

Tissue	Sample size	Yield (µg DNA)
Muscle (murine)	2 mg	1 µg
	5 mg	6 µg
		25 µg
Heart (murine)	5 mg	14 µg
	20 mg	45 µg
Kidney (canine)	5 mg	19 µg
	20 mg	56 µg
Lung (canine)	5 mg	16 µg
	20 mg	53 µg
Brain (canine)	5 mg	5 µg
	20 mg	30 µg
Mouse tails	0.5 cm	27 µg

Genomic DNA Isolation Protocol

Before beginning protocol

- Please read the entire protocol before beginning the isolation procedure.
- Preheat thermal rocker or rotator to 60°C. Alternatively, a water bath or heat block may be used, but additional handling will be required (see step 5).
- The first time the kit is used, prepare Solution 3 as follows: centrifuge the tube briefly before adding water to make sure the powder is at the bottom of the tube then add 540 µl of the provided sterile water. Store any unused Solution 3 at –20°C.
- Place tube with tissue sample in liquid nitrogen prior to weighing. Any unused sample should be placed back in liquid nitrogen until it can be returned to long-term storage

Extraction from Animal Tissue (2–50 mg)

1. Add 200 µl Solution 1, 40 µl Solution 2 and 16 µl Solution 3 to 1.5 ml microcentrifuge tube containing Resin (homogenization tube with Resin provided in kit).
2. Weigh out desired amount of tissue, chop into small pieces using a scalpel or razor blade. Place the tissue in the tube prepared in step 1.

Note: *Make sure the tissue is submerged in the solution.*

3. Homogenize the tissue thoroughly using pestle provided.
4. Add an additional 200 µl Solution 1 to each tube then mix by inverting 3–4 times.
5. Wrap lid of tube containing homogenized sample with Parafilm and incubate on thermal rocker or rotator at 60°C for 1 h. Alternatively, samples can be incubated in a water bath or heat block if the sample is inverted 2–3 times at 15 min intervals.

Note: *Samples can be incubated for up to 2 hours if necessary.*

Additional sterile water (not provided) is required for wetting the spin baskets

6. Centrifuge the tube briefly to collect condensation. Add 300 µl Solution 4 to each sample and well by inversion. Immediately spin tube at 14,000 × g for 15 min at room temperature.
7. Pre-wet spin filter (provided in kit) by pipetting 100 µl sterile water into filter insert and centrifuging spin filter for 1 min at 14,000 × g at room temperature. Discard water.
8. Transfer supernatant from step 6 to spin filter using a 1 ml pipet tip and centrifuge for 5 min 14,000 × g at room temperature. Note: If spin filter is clogged, transfer the unfiltered supernatant to a fresh spin filter and repeat steps 7 and 8 (3 extra spin filters are provided in kit). Combine the filtered flow through from both spin filters and proceed to step 9.
9. Transfer the flow through to a clean 1.5 ml microcentrifuge tube (the pellet is difficult to visualize in the original tube) then add 0.7 volumes isopropanol. Mix well by inversion then centrifuge 15 minutes, 14,000 × g at room temperature. Discard the supernatant. Note: If less than 10 mg tissue was used, a pellet may not be visible at this stage.
10. Wash pellet with 100 µl 70 % EtOH, spin 5 min, remove supernatant then air dry the pellet.

Note: *The pellet is very loose after this wash and care must be taken to avoid losing it.*

11. Using the large bore pipet tip provided, resuspend the dried pellet in 100 µl 1X TE for preps from 10–50 mg starting material or 20 µl for preps from < 10 mg starting material. Store the resuspended sample at –20°C. For best results, add the appropriate amount of TE, then begin resuspension by flicking the tube gently. Complete resuspension by pipetting up and down with the tip provided. If RNA-free genomic DNA is required, add RNase A to a final concentration of 20 µg/ml. RNA may interfere with some enzymatic reactions, but will not inhibit PCR.

United States & Canada **800-207-0144**
Germany **0800 6931 000**
United Kingdom **0800 622935**
Or your local sales office

Troubleshooting guide

Problem	Possible causes	Solution
Low or no yield	Insufficient homogenization	Chop tissue into small pieces then grind until completely homogenized.
	Insufficient Solution 3	Make sure all of provided powder is resuspended before use.
		If precipitate forms in Solution 3, incubate at 37°C until dissolved.
	Lost pellet during EtOH wash	Be careful not to aspirate pellet when removing EtOH.
Clogged spin filter	Tissue rich in protein	Use extra spin filter provided See step 8
Low MW DNA	Vortex mixer used	Gently invert sample to mix
	Used small bore pipet tips	Use only 1 ml pipet tips and large bore tips provided
Incomplete digest of DNA using restriction enzymes	DNA contains impurities inhibiting digest	Perform additional purification step using phenol/chloroform
Leakage of sample during 60°C incubation	Homogenization resin may be on tube lip preventing complete closure of cap	Wipe tube lip before closing cap.
Degraded genomic DNA	DNase activity digested genomic DNA	Make sure tissue is fully submerged if there will be a delay before homogenization.
		Check whether the correct amount of solutions 2 and 3 were added.
		Step 5 was performed at < 55°C.
		Tissue was stored above –70°C.
	Sample was vortexed during isolation procedure.	Always mix by inverting the tube, not vortexing.
Spill over of sample during homogenization	Too much liquid in tube	Reduce volume of liquid added before homogenizing tissue. Add remainder following homogenization.

Related Products and Separate Components

Product		Size	Cat. No.
Pellet Paint™ Co-Precipitant		125 rxn	69049-3
p53-Prime® II Genomic Kit		100 rxn	69881-3
MagPrep™ Blood Genomic Kit		100 rxn	70690-3
Perfect DNA™ Markers	0.5–12 kbp	100 lanes	69002-3
	0.1–12 kbp		70087-3
	0.05–10 kbp		70540-3