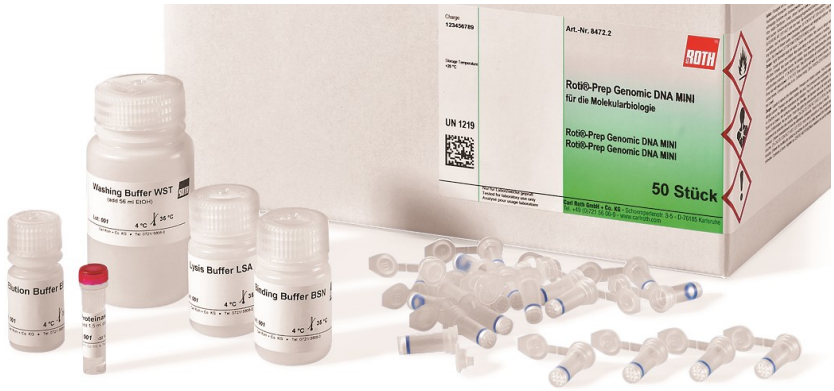
Instructions for use

ROTI®Prep Genomic DNA MINI

8472

For isolation of genomic DNA from tissues, rodent tails, FFPE tissues, buccal swabs, cell cultures



Introduction and product description:

- Preparation by well-known mini spin-column system
- Fast, easy and efficient
- Preparation in approx. 15 minutes (excluding lysis)
- Yield up to 65 µg gDNA, depending on source material

ROTI®Prep Genomic DNA MINI has been developed for the isolation of genomic DNA from various sources.

The isolation is carried out through the proven and reliable spin column technique, which is very easy to handle and needs only few steps.

In addition to the especially on proteinase K adapted lysis buffer, the filtration spin column has been optimised, permitting particularly high extraction rates. Extraction from up to 40 mg tissue, therefore, allows a yield of up to 65 µg gDNA. The thereby extracted DNA is free of RNA and proteins, and can directly be stored or used for downstream applications.

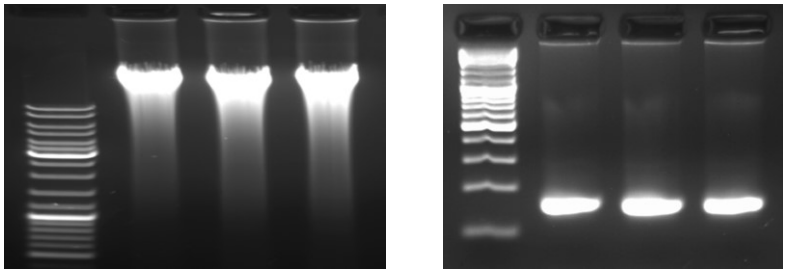


Figure: Typical test gel following isolation of genomic DNA (A) and subsequent GAPDH-PCR (B) from rodent tail (frozen, 8 mm each).
Yield of gDNA (from left to right): 22.6 µg, 27.7 µg and 28 µg. Ratio A_{260}:A_{280} = 1.97±0.01.

Suitable source material:

- Tissue (up to 40 mg)
- Rodent tails (0.5 – 1 cm)
- FFPE tissue (paraffin embedded tissue)
- Buccal swabs
- Cell culture (up to 5x10^6 cells)

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Caution:

*Lysis Buffer LSA**	**Warning**	H319
Binding Buffer BSN	**Danger**	H225-H319-H336
Proteinase K	**Danger**	H315-H319-H334-H317-H335

*In case of forming of precipitates, please dissolve by careful warming.

MSDS: the appropriate MSDS can be downloaded from our website www.carlroth.com.

All due care and attention should be exercised in handling the materials and reagents contained in the kit. Always wear gloves while handling these reagents and avoid any skin contact! In case of contact, flush eyes or skin with a large amount of water immediately.

Literature Citation: When describing a procedure for publication using this product, please refer to it as *Carl Roth's ROTI®Prep Genomic DNA MINI Kit*.

1. Materials provided with the kit and storage conditions

Amount	Component	Storage
6 / 30 / 5x30 mg	Proteinase K	+4 °C / -20 °C*
10 / 25 / 120 ml	Lysis Buffer LSA	>20 °C**
16 / 16 / 60 ml	Binding Buffer BSN	RT
6 / 24 / 2x60 ml	Washing Buffer WST (Base)	RT
2x2 / 15 / 2x30 ml	Elution Buffer EB	RT
10 / 50 / 250	Mini spin columns (blue)	RT
20/ 100 / 500	1.5 ml Elution tubes	RT
10 / 50 / 250	2 ml Collection tubes	RT

* Lyophilized Proteinase K should be stored at +4 °C. Prior to use, dissolve Proteinase K in 0.3 / 1.5 / 5x1.5 ml sterile, distilled water as given below. Dissolved Proteinase K should be stored in aliquots at -20 °C. Avoid repeated freeze&thaw cycles for each tube.

** Lysis Buffer should be stored at over 20 °C in order to prevent precipitation of reagents.

The ROTI®Prep Genomic DNA MINI Kit should be stored dry, at room temperature (14-25 °C). Before every use make sure that all components have room temperature.
If there are any precipitates within the provided solutions solve these precipitates by careful warming. This kit is stable for at least 6 months after receipt, when stored as directed.

Contents of this Kit may not be bought separately.

The user is responsible to validate the performance of the ROTI®Prep Genomic DNA MINI Kit for any particular use, since the performance characteristics of our kits have not been validated for any specific application.

2. Required Material and Equipment not included in this kit

- Ethanol 96-99.8 %, e.g. Ethanol p.a. (e.g. 9065.1)
- Distilled, sterilized H_2O
- RNAse A, stock solution 100 mg/ml (e.g. 7156.1, 100 mg solubilized in 1 ml distilled water, made DNAse-free by cooking)
- Xylene or octane (optional)

3. Application

ROTI®Prep Genomic DNA MINI is designed for isolation of high-purity genomic DNA from various source material. Spin column based preparation allows elution in a small volume of low-salt buffer, eliminating time-consuming phenol-chloroform extraction or alcohol precipitation. The columns may be used either in micro-centrifuges or on vacuum manifolds.

The kit allows the extraction of **up to 65 µg** gDNA per preparation from up to 40 mg of rodent tails (0.5 – 1 cm), paraffin embedded tissue, buccal swabs or cultured cells (up to $5x10^6$).

During lysis, the sample has to be mixed carefully and, if possible, constantly. Reduced movement of the lysis mix will reduce the lysis efficiency, and, subsequently, the recovery rate of gDNA. We recommend using a shaking platform or thermomixer in order to keep the sample at constant movement.

When elution DNA from buccal swabs, we recommend to keep the swab in the tube during the whole lysis procedure, in order to achieve a maximum yield of DNA. In order to enable closing of the cap the shaft may be cut, however. Removal of the swab from the tube ahead of time will lead to a dramatically reduced yield!

Repetition of elution (final step) often enhances the recovery rate overall. However, even so often, concentration of the recovered gDNA is reduced in parallel, since the whole amount of buffer used for elution is higher. Elution is 100 µl Elution Buffer EB, followed by another elution step in 100 µl Elution Buffer EB, may be recommended.

Store the extracted DNA at +4 °C. For long time storage placing at -20 °C is recommended.

For centrifugation we recommend a standard microcentrifuge. Centrifugation steps should be carried out at room temperature.

Avoid repeated freeze&thaw cycles for the tissue to be extracted.

Before start, be sure to

- Add Ethanol (96-99.8 %, not incl. in this kit) to the Washing Buffer WST as follows:
  8472.1 (10 Preps): 14 ml (20 ml final vol.)
  8472.2 (50 Preps): 56 ml (80 ml final vol.)
  8472.3 (250 Preps): 140 ml to each bottle (2x 200 ml final vol.)
  Mix thoroughly and keep the bottle always firmly closed!
- Dissolve Proteinase K by addition of distilled H_2O as follows:
  8472.1 (10 Preps): 0.3 ml
  8472.2 (50 Preps): 1.5 ml
  8472.3 (250 Preps): 1.5 ml to each tube
  Mix thoroughly!
- Heat one thermal mixer or water bath to 50 °C.
- For elution from paraffin embedded tissue: be prepared to heat another thermal mixer or water bath to 90 °C.

Please note: Centrifugation steps should be carried out at room temperature

4. Workflow

The workflow has been provided on a separate page, in order to use a copy for in-process protocol or filing in your personal lab files.

4.1 DNA isolation from tissue samples or rodent tails (max. 10 mg tissue or 1 cm tail section)

Step (RT = room temperature)	**done**

1. Tissue lysis	
Cut max. 40 mg of tissue sample into small pieces and place the tissue into a 1.5 ml or 2.0 ml reaction tube.	
Add 400 µl **Lysis Buffer LSA**, 25 µl Proteinase K solution, and 3 µl RNase A (stock solution 100 mg/ml; not included in the kit). Mix vigorously by pulsed vortexing for 5 sec.	
Incubate at 50 °C under constant agitation* until the sample is *completely* lysed, approx. 0.5-2 h for tissue sample and 3 h for rodent tails.**	
After lysis has been completed, centrifuge the lysed sample at 10.000 g (or 12.000 rpm) for 30 sec to spin down unlysed material.	
Transfer the supernatant to a fresh 1.5 ml reaction tube.	
Add 200 µl **Binding Buffer BSN** to the supernatant. Mix by vortexing or pipetting until a *homogenous* solution is achieved.	

2. Column Loading	
Place each Spin Column into a 2 ml collection tube	
Apply the mix of supernatant/Binding Buffer BSN to the spin column.	
Centrifuge at 10.000 g (or 12.000 rpm) for 2 min/RT and discard the flow-through.***	

3. Column Washing	
Add 700 µl of **Washing Buffer WST** to the Spin Column	
Centrifuge at 10.000 g (or 12.000 rpm) for 1 min/RT and discard the flow-through***	
Add 700 µl of **Washing Buffer WST** to the Spin Column	
Centrifuge at 10.000 g (or 12.000 rpm) for 1 min/RT and discard the flow-through***	
Again centrifuge columns at 12.000-14.000 rpm (or full speed) for 2 mins/RT in order to remove residual ethanol.	

4. Elution	
Place the Spin Column into a clean 1.5 ml elution tube	
Add 200 µl **Elution Buffer EB** to the centre of the membrane****	
Incubate for 1 min at room temperature	
Centrifuge at 6.000 g (or 8.000 rpm) for 1 min/RT to elute DNA	

* We recommend using a shaking platform or thermomixer for continuous agitation of the sample. Alternatively, vortex the sample 3-4 times during incubation. Agitation is vital for a decent recovery rate.

** Check lysis efficiency before proceeding in the protocol. If the lysis is not complete, prolong the incubation time in Lysis Buffer LSA. This step is critical, don't be impatient.

*** If the solution has not completely passed through the spin column, centrifuge again at higher speed or prolong the centrifugation time.

**** Elution with lower volumes of Elution Buffer (e.g. 100 µl Elution Buffer only) increases the final concentration of DNA. Repetition of the elution step often increases the yield of gDNA overall, but may reduce the concentration after pooling of the fractions.

4.2 DNA isolation from paraffin embedded tissue samples

Step (RT = room temperature)	**done**

1. Deparaffination	
Place the sample into a 2.0 ml tube, add 1 ml Octane or Xylene. Vortex carefully to dissolve the paraffin. Monitor this process carefully until the tissue sample looks transparent (while paraffin remains white).	
Centrifuge at max. speed for 5 mins/RT. Discard the supernatant very carefully by pipetting. *Do not remove the pellet*.	
Check the sample carefully and repeat the last step until the sample is *completely* transparent.	
Add 1 ml ethanol (96-99.8 %) to the pellet and vortex vigorously.	
Centrifuge at maximum speed for 3 min/RT and remove the ethanol by pipetting. *Do not remove the pellet*.	
Add 1 ml ethanol (96-99.8 %) to the pellet and vortex vigorously.	
Centrifuge at maximum speed for 3 min/RT and remove the ethanol by pipetting. *Do not remove the pellet*.	
Incubate the open tube at 37 °C for 10-15 mins to evaporate the residual ethanol.	

2. Tissue lysis	
Add 400 µl **Lysis Buffer LSA,** 25 µl Proteinase K solution, and 3 µl RNase A (stock solution 100 mg/ml; not included in the kit). Mix vigorously by pulsed vortexing for 5 sec.	
Incubate at 50 °C under constant agitation* until the sample is *completely* lysed, approx. 0.5-2 h for tissue samples.**	
After lysis has been completed, place the sample into a thermomixer prewarmed at 90 °C and incubate the sample for 1 h.***	
Add 200 µl **Binding Buffer BSN** to the sample. Mix by vortexing or pipetting until a *homogenous* solution is achieved.	

3. Column Loading	
Place each Spin Column into a 2 ml collection tube	
Apply the mix of sample/Binding Buffer BSN to the spin column.	
Centrifuge at 10.000 g (or 12.000 rpm) for 2 min/RT and discard the flow-through.****	

4. Column Washing	
Add 700 µl of **Washing Buffer WST** to the Spin Column	
Centrifuge at 10.000 g (or 12.000 rpm) for 1 min/RT and discard the flow-through****	
Add 700 µl of **Washing Buffer WST** to the Spin Column	
Centrifuge at 10.000 g (or 12.000 rpm) for 1 min/RT and discard the flow-through****	
Again centrifuge columns at 12.000-14.000 rpm (or full speed) for 2 mins/RT in order to remove residual ethanol.	

Sequel: DNA isolation from paraffin embedded tissue samples

Step (RT = room temperature)	**done**

5. Elution	
Place the Spin Column into a clean 1.5 ml elution tube	
Add 200 µl **Elution Buffer EB** to the centre of the membrane*****	
Incubate for 1 min at room temperature	
Centrifuge at 6.000 g (or 8.000 rpm) for 1 min/RT to elute DNA	

* We recommend using a shaking platform or thermomixer for continuous agitation of the sample. Alternatively, vortex the sample 3-4 times during incubation. Agitation is vital for a decent recovery rate.
** Check lysis efficiency before proceeding in the protocol. If the lysis is not complete, prolong the incubation time in Lysis Buffer LSA. This step is critical, don't be impatient. During lysis, make sure to heat up a thermomixer or water bath to 90 °C.
*** Only place the sample into the thermomixer when the temperature has reached 90 °C !!!
**** If the solution has not completely passed through the spin column, centrifuge again at higher speed or prolong the centrifugation time.
***** Elution with lower volumes of Elution Buffer (e.g. 100 µl Elution Buffer only) increases the final concentration of DNA. Repetition of the elution step often increases the yield of gDNA overall, but may reduce the concentration after pooling of the fractions.

4.3 DNA isolation from buccal swabs

For a maximum yield of DNA, the swab has to remain in the tube during the whole lysis procedure. We recommend to cut the shaft of the swab, in order to enable closing of the cap. Removal of the swab from the tube ahead of time will lead to a dramatically reduced yield!

Step (RT = room temperature)	**done**

1. Tissue/cell lysis	
Place the swab into a 1.5 ml or 2.0 ml reaction tube and cut the shaft.	
Add 400 µl **Lysis Buffer LSA**, 25 µl Proteinase K solution, and 3 µl RNase A (stock solution 100 mg/ml; not included in the kit). Mix vigorously by pulsed vortexing for 5 sec.	
Incubate at 50 °C under constant agitation* for 10-15 mins.	
Remove the swab by pressing the tip of the swab to the inner tube wall, in order to quantitatively squeezing the liquid from the cotton wool into the tube.	
Add 200 µl **Binding Buffer BSN** to the sample. Mix by vortexing or pipetting in order to achieve a homogenous solution.	

2. Column Loading	
Place each Spin Column into a 2 ml collection tube	
Apply the mix of sample/Binding Buffer BSN to the spin column.	
Centrifuge at 10.000 g (or 12.000 rpm) for 2 min/RT and discard the flow-through.**	

3. Column Washing	
Add 700 µl of **Washing Buffer WST** to the Spin Column	
Centrifuge at 10.000 g (or 12.000 rpm) for 1 min/RT and discard the flow-through**	
Add 700 µl of **Washing Buffer WST** to the Spin Column	
Centrifuge at 10.000 g (or 12.000 rpm) for 1 min/RT and discard the flow-through**	
Again centrifuge columns at 12.000-14.000 rpm (or full speed) for 2 mins/RT in order to remove residual ethanol.	

4. Elution	
Place the Spin Column into a clean 1.5 ml elution tube	
Add 200 µl **Elution Buffer EB** to the centre of the membrane****	
Incubate for 1 min at room temperature	
Centrifuge at 6.000 g (or 8.000 rpm) for 1 min/RT to elute DNA	

* We recommend using a shaking platform or thermomixer for continuous agitation of the sample. Alternatively, vortex the sample 3-4 times during incubation. Agitation is vital for a decent recovery rate.
** If the solution has not completely passed through the spin column, centrifuge again at higher speed or prolong the centrifugation time.
*** Elution with lower volumes of Elution Buffer (e.g. 100 µl Elution Buffer only) increases the final concentration of DNA. Repetition of the elution step often increases the yield of gDNA overall, but may reduce the concentration after pooling of the fractions.

4.4 DNA isolation from cultured cells (5 x 10^6 cells in maximum)

Adherent cells should be loosened from the culture bottle surface by trypsination or EDTA. Remove cells and wash in PBS. Count your cell number and select the appropriate cell amount, 5x10^6 cells in maximum*

Step (RT = room temperature)	**done**

1. Cell lysis	
Pellet cells by centrifugation at 5.000 g (or 7.500 rpm) for 10 mins and discard the supernatant.*	
Add 200 µl **Lysis Buffer LSA**, 25 µl Proteinase K solution, and 3 µl RNase A (stock solution 100 mg/ml; not included in the kit). Mix vigorously by pulsed vortexing for 5 sec.	
Incubate at 50 °C under constant agitation* until the sample is *completely* lysed, approx. 30 mins.**	
Add 200 µl **Binding Buffer BSN** to the sample. Mix by vortexing or pipetting until a *homogenous* solution is achieved.	

2. Column Loading	
Place each Spin Column into a 2 ml collection tube	
Apply the mix of sample/Binding Buffer BSN to the spin column.	
Centrifuge at 10.000 g (or 12.000 rpm) for 2 min/RT and discard the flow-through.***	

3. Column Washing	
Add 700 µl of **Washing Buffer WST** to the Spin Column	
Centrifuge at 10.000 g (or 12.000 rpm) for 1 min/RT and discard the flow-through***	
Add 700 µl of **Washing Buffer WST** to the Spin Column	
Centrifuge at 10.000 g (or 12.000 rpm) for 1 min/RT and discard the flow-through***	
Again centrifuge columns at 12.000-14.000 rpm (or full speed) for 2 mins/RT in order to remove residual ethanol.	

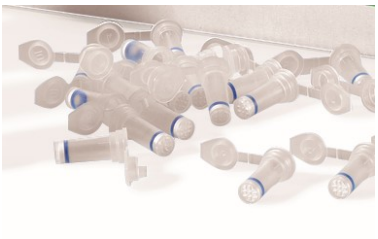
4. Elution	
Place the Spin Column into a clean 1.5 ml elution tube	
Add 200 µl **Elution Buffer EB** to the centre of the membrane****	
Incubate for 1 min at room temperature	
Centrifuge at 6.000 g (or 8.000 rpm) for 1 min/RT to elute DNA	

* Alternatively, adherent cells may be incubated in 96wells. Wash cells with PBS and perform the lysis in the 96well under agitation. Following lysis, the cell solution has to be transferred into 1.5 ml tubes for centrifugation.
** We recommend using a shaking platform or thermomixer for continuous agitation of the sample. Alternatively, vortex the sample 3-4 times during incubation. Agitation is vital for a decent recovery rate.
*** If the solution has not completely passed through the spin column, centrifuge again at higher speed or prolong the centrifugation time.
**** Elution with lower volumes of Elution Buffer (e.g. 100 µl Elution Buffer only) increases the final concentration of DNA. Repetition of the elution step often increases the yield of gDNA overall, but may reduce the concentration after pooling of the fractions.

5. Trouble Shooting

Problem / probable cause	Comments and suggestions
Clogged spin filter	
Insufficient lysis and/or too much starting material	Increase lysis time.
	Increase centrifugation speed.
	After lysis centrifuge the lysate to pellet unlysed material.
	Check storage conditions and usage of Proteinase K. Optionally replace Proteinase K by a fresh lot.
	Reduce amount of starting material. Overload of filters reduces yield.
Low recovery	
Insufficient lysis	See above
Insufficient mixing with Binding Buffer BSN	Mix sample very well with Binding Buffer BSN by pipetting or by vortexing prior to transfer of the sample onto the Spin Filter
Low amount of recovered gDNA.	Add the Elution Buffer directly onto the centre of the Spin Column.
	Prolong the incubation time with Elution Buffer.
	Increase volume of Elution Buffer used or repeat elution step.
Low concentration of eluted gDNA	Reduce the volume of Elution Buffer used. Optionally: prolong elution / incubation time.
Degraded or sheared DNA	
Incorrect storage of starting material.	Make sure that the starting material is frozen immediately in liquid N_2 or in minimum at -20 °C, and is stored continuously at -80 °C!
	Avoid repeated freezing and thawing of the starting material.
Old material	Use fresh material. Check and improve storage conditions.
RNA contamination	
Unsufficient RNAse digestion during lysis	Add RNAse A during lysis.
	Check storage conditions and usage of RNAse A. Optionally replace RNAse A by a fresh lot.

Ordering information:
(for detailed kit content see Table under 1.)



ROTI®Prep Genomic DNA MINI	**10 preps (Mini kit)**	**8472.1**
ROTI®Prep Genomic DNA MINI	**50 preps (Kit)**	**8472.2**
ROTI®Prep Genomic DNA MINI	**250 preps (Maxi kit)**	**8472.3**

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