Instructions for use

ROTI®Prep DNA Micro

20H5

Kit for isolation of genomic DNA from small sample volumes (animal tissue, cells, blood.

1. Introduction and product description

- Preparation by well-known mini spin-column system
- Fast, easy and efficient
- Preparation in approx. 8 minutes (excluding lysis)
- Average Ratio A_{260}:A_{280}: 1.7-2.0

ROTI®Prep DNA Micro was developed to isolate genomic DNA from small sample amounts of different source materials like micro biopsies, whole blood up to 50 µl, blood sticks and from limited amounts of cells.

The isolation is based on a new method and combines extremely fast sample lysis with the subsequent binding of the DNA to the surface of a column membrane. After several washing and centrifugation steps, the DNA is eluted from the membrane using an elution buffer. The extracted DNA is of high quality and suitable for all common downstream applications.

Suitable source material:

- Cell remnants or small tissue samples (up to 5 mg)
- Eucaryotic cells (up to $1x10^6$)
- Blood samples (up to 50 µl) and Bloodsticks
- Paraffin embedded tissue samples

2. Product use and warranty

2.1 Intended Use

- For research use only.

- Please read the instructions for use carefully and follow the instructions accordingly. Reliability of results cannot be guaranteed if there are any deviations in the procedure.

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

2.2 Safety Precautions

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.

Caution:

Lysis Buffer LSM  Danger H318-H302-H315-H412-H319

Binding Buffer BSM  Danger H225-H319-H336

Proteinase K*  Danger H315-H319-H334-H317-H335

Washing Buffer WSA  Danger H302-H312-H332-H314-H412

Attention: Do not add bleach or acidic components to the waste after sample preparation!

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

3. Materials provided in this kit and storage conditions

3.1. Included Kit components

Amount	Component	Storage
2x 2 / 15 ml	Lysis Buffer LSM	RT
2x 2 / 15 ml	Binding Buffer BSM	RT
For 1x 0.3/ 1x 1.5 ml	Proteinase K*	+4 °C
3 / 15 ml	Washing Buffer WSA (conc.)	RT
3 / 15 ml	Washing Buffer WST (conc.)	RT
2 / 10 ml	Elution Buffer EB	RT
10 / 50	Mini spin columns	RT
10 / 50	1.5 ml Elution tubes	RT
30 / 3x 50	2 ml Collection tubes	RT

- Lyophilized **Proteinase K** should be stored at +4 °C. Prior to use, dissolve Proteinase K in 1x 0.3 / 1x 1.5 ml sterile, distilled water as given below. Dissolved Proteinase K should be stored in aliquots at 4-8 °C. Avoid repeated freeze&thaw cycles for each tube.

The ROTI®Prep DNA Micro 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 DNA Micro Kit for any particular use, since the performance characteristics of our kits have not been validated for any specific application.

3.2. Required Material and Equipment not included in this kit

- Ethanol 96-99.8 %, e.g. Ethanol p.a. (e.g. 9065.1)
- Sterile, nuclease-free ddH_2O (e.g. 1HPE.1)
- RNase A (100 mg/ml, e.g. 7156.1)

4. Application

ROTI®Prep DNA Micro Kit is designed for isolation of high-purity genomic DNA from small quantity samples. 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 high quality DNA from Cell remnants or small tissue samples (up to 5 mg), eucaryotic cells (up to $1x10^6$), blood sticks and whole blood samples (up to 50 µl) and paraffin embedded tissue samples.

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.

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.

4.1. Before start, be sure to…

- **Add Ethanol** (96-99.8 %, not incl. in this kit) to the Washing Buffer WSA (conc.) as follows:
  20H5.1 (10 Preps): 3 ml (6 ml final vol.)
  20H5.2 (50 Preps): 15 ml (30 ml final vol.)
  Mix thoroughly and keep the bottle always firmly closed!
- **Add Ethanol** (96-99.8 %, not incl. in this kit) to the Washing Buffer WST (conc.) as follows:
  20H5.1 (10 Preps): 7 ml (10 ml final vol.)
  20H5.2 (50 Preps): 35 ml (50 ml final vol.)
  Mix thoroughly and keep the bottle always firmly closed!
- **Dissolve Proteinase K** by addition of distilled H_2O as follows:
  20H5.1 (10 Preps): 0.3 ml
  20H5.2 (50 Preps): 1.5 ml
  Mix thoroughly!
- Heat one thermal mixer or water bath to 56 or 60 °C.
- Pre-fill the needed amount of RNase free water or Elution Buffer into a 2.0 ml reaction tube and incubate at 60 °C until the elution step.
- For Lysis steps we recommend using a shaking platform (thermal mixer, water bath or another rocking platform) for a continuous shaking of the sample. Alternatively, vortex the sample 3–4 times during the incubation. No shaking will reduce the lysis efficiency.

4.2. 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.

4.2.1 DNA isolation from tissue samples or cell remnants

Step (RT = room temperature)	done

1. Cell lysis	
Cut max. 5 mg of tissue sample into small pieces and place the tissue into a 1.5 ml reaction tube.	
Add 200 µl **Lysis Buffer LSM** and 20 µl **Proteinase K** and mix vigorously by pulsed vortexing for 5 s. Incubate at 50 °C until the sample is completely lysed (appr. 15 – 30 minutes)	
The kit co-purifies DNA and RNA. If RNA-free genomic DNA is required, add 4 µl of a **RNase A** stock solution (100 mg/ml) to the sample, vortex shortly and incubate for 5 minutes at room temperature.	
Add 200 µl **Binding Buffer BSM** to the sample. Mix by vortexing carefully 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 BSM to the spin column.	
Centrifuge at 11.000 g (or 13.000 rpm) for 2 min/RT and discard the flow-through and the collection tube***	

3. Column Washing	
Place the Spin Column into a new 2 ml collection tube	
Add 400 µl of **Washing Buffer WSA** to the Spin Column	
Centrifuge at 11.000 g (or 13.000 rpm) for 1 min/RT and discard the flow-through and the collection tube	
Place the Spin Column into a new 2 ml collection tube	
Add 750 µl of **Washing Buffer WST** to the Spin Column	
Centrifuge at 11.000 g (or 13.000 rpm) for 1 min/RT and discard the flow-through and the collection tube	
Place the Spin Column into a new 2 ml collection tube	
Again centrifuge columns at 12.000-14.000 rpm (or full speed) for 3 mins/RT in order to remove residual ethanol.	

4. Elution	
Place the Spin Column into a clean 1.5 ml elution tube	
Add 50-100 µl **Elution Buffer EB** to the centre of the membrane	
Incubate for 2 min at room temperature	
Centrifuge at 11.000 g (or 13.000 rpm) for 1 min/RT to elute DNA	
Repeat the elution step (by loading the eluted DNA again on the spin column) to increase the yield of extracted DNA	

4.2.2 DNA isolation from eucaryotic cell cultures

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 LSM** and 20 µl **Proteinase K** and mix vigorously by pulsed vortexing for 5 s. Incubate at 50 °C until the sample is completely lysed (appr. 15 – 30 minutes depends on number of cells).	
The kit co-purifies DNA and RNA. If RNA-free genomic DNA is required, add 4 µl of a RNase A stock solution (100 mg/ml) to the sample, vortex shortly and incubate for 5 minutes at room temperature.	
Add 200 µl **Binding Buffer BSM** 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 BSM to the spin column.	
Centrifuge at 11.000 g (or 13.000 rpm) for 2 min/RT and discard the flow-through and the collection tube***	

3. Column Washing	
Place the Spin Column into a new 2 ml collection tube	
Add 400 µl of **Washing Buffer WSA** to the Spin Column	
Centrifuge at 11.000 g (or 13.000 rpm) for 1 min/RT and discard the flow-through and the collection tube***	
Place the Spin Column into a new 2 ml collection tube	
Add 750 µl of **Washing Buffer WST** to the Spin Column	
Centrifuge at 11.000 g (or 13.000 rpm) for 1 min/RT and discard the flow-through and the collection tube***	
Place the Spin Column into a new 2 ml collection tube	
Again centrifuge columns at 12.000-14.000 rpm (or full speed) for 3 mins/RT in order to remove residual ethanol.	

4. Elution	
Place the Spin Column into a clean 1.5 ml elution tube	
Add 50-100 µl **Elution buffer EB** to the centre of the membrane****	
Incubate for 2 min at room temperature	
Centrifuge at 11.000 g (or 13.000 rpm) for 1 min/RT to elute DNA	
Repeat the elution step (by loading the eluted DNA again on the spin column) to increase the yield of extracted DNA	

4.2.3 DNA isolation from blood samples

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

1. Cell lysis	
Pipette 50 µl of whole blood sample (or at least 1µl) into a 1.5 ml reaction tube.	
Add 250 µl **Lysis Buffer LSM** and 20 µl **Proteinase K**, mix vigorously by pulsed vortexing for 10 seconds and incubate the sample at 70 °C for 10 minutes.	
The kit co-purifies DNA and RNA. If RNA-free genomic DNA is required, add 4 µl of a **RNase A** stock solution (100 mg/ml) to the sample, vortex shortly and incubate for 5 minutes at room temperature.	
Add 200 µl **Binding Buffer BSM** to the sample. Mix by sorrowly pipetting up and down 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 to the spin column.	
Centrifuge at 11.000 g (or 13.000 rpm) for 1 min/RT and discard the flow-through.	

3. Column Washing	
Place the Spin Column into a new 2 ml collection tube	
Add 400 µl of **Washing Buffer WSA** to the Spin Column	
Centrifuge at 11.000 g (or 13.000 rpm) for 30 sec/RT and discard the flow-through and the collection tube	
Place the Spin Column into a new 2 ml collection tube	
Add 750 µl of **Washing Buffer WST** to the Spin Column	
Centrifuge at 11.000 g (or 13.000 rpm) for 1 min/RT and discard the flow-through and the collection tube***	
Place the Spin Column into a new 2 ml collection tube	
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 50-100 µl **Elution buffer EB** to the centre of the membrane	
Incubate for 2 min at room temperature	
Centrifuge at 11.000 g (or 13.000 rpm) for 1 min/RT to elute DNA	
Repeat the elution step (by loading the eluted DNA again on the spin column) to increase the yield of extracted DNA.	

4.2.4 DNA isolation from blood sticks

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

1. Cell lysis	
Place the blood stick into a 1.5 ml reaction tube.	
Add 200 µl **Lysis Buffer LSM** and 20 µl **Proteinase K** and mix vigorously by pulsed vortexing for 5 s. Incubate at 50 °C under continuous shaking for 30 min.	
Add 200 µl **Binding Buffer BSM** to the sample. Mix carefully 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 BSM to the spin column.	
Centrifuge at 11.000 g (or 13.000 rpm) for 1 min/RT and discard the flow-through and the collection tube	

3. Column Washing	
Place the Spin Column into a new 2 ml collection tube	
Add 400 µl of **Washing Buffer WSA** to the Spin Column	
Centrifuge at 11.000 g (or 13.000 rpm) for 30 sec/RT and discard the flow-through and the collection tube	
Place the Spin Column into a new 2 ml collection tube	
Add 750 µl of **Washing Buffer WST** to the Spin Column	
Centrifuge at 11.000 g (or 13.000 rpm) for 30 sec/RT and discard the flow-through and the collection tube	
Place the Spin Column into a new 2 ml collection tube	
Again centrifuge columns at 12.000-14.000 rpm (or full speed) for 3 mins/RT in order to remove residual ethanol.	

4. Elution	
Place the Spin Column into a clean 1.5 ml elution tube	
Add 50-100 µl **Elution buffer EB** to the centre of the membrane	
Incubate for 2 min at room temperature	
Centrifuge at 11.000 g (or 13.000 rpm) for 1 min/RT to elute DNA	
Repeat the elution step (by loading the eluted DNA again on the spin column) to increase the yield of extracted DNA	

4.2.5 DNA isolation from paraffin embedded tissues

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

1. Cell lysis	
Place a piece of starting material into a 2.0 ml reaction tube	
Add 1 ml **Octane or Xylene** and vortex carefully to dissolve the paraffin. Follow the dissolution until the tissue sample looks transparent (while paraffin remains white).	
Centrifuge at max. speed for 5 min/RT. Carefully discard the supernatant using a pipette. Repeat this step if any paraffin is still in the sample.	
Add 1 ml **ethanol** to the pellet and vortex vigorously.	
Centrifuge at max. speed for 3 min/RT. Carefully remove the ethanol using a pipette. Repeat the ethanol washing step	
Incubate the open tube at 37 °C for 10–15 min to evaporate the residual ethanol completely.	
Add 200 **Lysis Buffer LSM** and 20 µl **Proteinase K,** mix vigorously by pulsed vortexing for 5 sec and incubate the sample at 50 °C until the sample is completely lysed	
Pre-heat the thermal mixer without the sample to 90 °C, afterwards incubate the lysed sample for 60 min at 90 °C	
Add 200 µl **Binding Buffer BSM** to the sample. Mix by sorrowly pipetting up and down 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 BSM to the spin column.	
Centrifuge at 11.000 g (or 13.000 rpm) for 1 min/RT and discard the flow-through. Repeat this step with the rest of you sample (only necessary in case of 400 µl blood sample).	

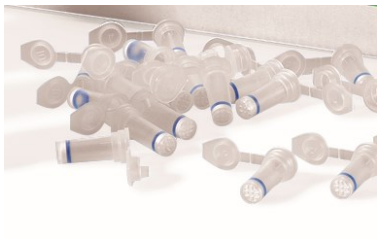
3. Column Washing	
Place the Spin Column into a new 2 ml collection tube	
Add 400 µl of **Washing Buffer WSA** to the Spin Column	
Centrifuge at 11.000 g (or 13.000 rpm) for 30 sec/RT and discard the flow-through and the collection tube	
Place the Spin Column into a new 2 ml collection tube	
Add 750 µl of **Washing Buffer WST** to the Spin Column	
Centrifuge at 11.000 g (or 13.000 rpm) for 30 sec/RT and discard the flow-through and the collection tube	
Place the Spin Column into a new 2 ml collection tube	
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 50-100 µl **Elution buffer EB** to the centre of the membrane	
Incubate for 2 min at room temperature	
Centrifuge at 11.000 g (or 13.000 rpm) for 1 min/RT to elute DNA	
Repeat the elution step (by loading the eluted DNA again on the spin column) to increase the yield of extracted DNA.	

5. Trouble Shooting

Problem / probable cause	Comments and suggestions
1. 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.
2. 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.
3. 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.
4. 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 DNA Micro	**10 preps**	**20H5.1**
ROTI®Prep DNA Micro	**50 preps**	**20H5.2**
ROTI®Prep DNA Micro	**250 preps**	**20H5.3**

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