Instructions for use

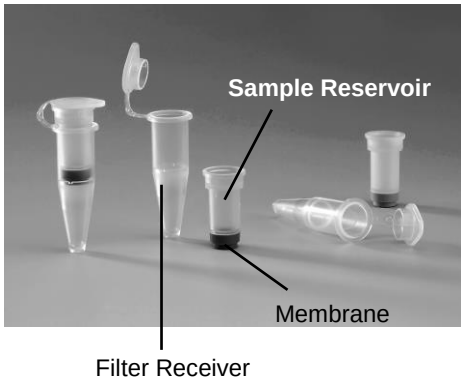
ROTI®Spin MINI

CL12.1, CL13.1, CL14.1, CL15.1

- Simple, reliable concentrating and desalting of 50 to 500 µL samples in just 5 to 15 minutes
- Rapid processing of samples.
- Typical recoveries are greater than 90% (if MWCO is chosen appropriately)
- Provided with specially designed low protein-binding membrane.
- MWCOs color-coded for easy identification.
- Constructed of low binding polypropylene.
- Ultrasonically welded seals prevent bypass or seal failure.
- Fits standard centrifuge rotors that accept 1.5 ml tubes

I. Principle of function

Ultrafiltration, caused by centrifugation. Centrifugal force moves the sample toward the membrane and presses all molecules small enough through the pores.
Biomolecules larger than the nominal molecular weight cut off (MWCO) of the membrane are retained in the sample reservoir, while solvents and molecules of lower molecular weight are collected at the bottom of the filtrate receiver.
Please note: the MWCO does not function as a sharp separation border, but is defined by its ability to retain 90 % of an ideally globular molecule. Many factors, including ionic conditions, interactions between molecules, and tertiary structure, can affect the retention of biomolecules. We recommend that you pre-test retentivity of your biomolecular solution.



II. Applications (examples)

- Concentrate, purify, and desalt peptides, proteins, oligonucleotides, DNA, and RNA.
- Clean up labelling and PCR reactions, e.g. removal of non-incorporated nucleotides.
- Separate proteins, oligonucleotides, and RNA from acrylamide gels.
- Sample preparation for HPLC analysis.

III. Physical Parameters:

Filter Media: modified polyethersulfone on polyethylene substrate with low protein-binding
Filtrate Receiver: Polypropylene
Effective Filtration Area: 0.28 cm^2
Operating Temperature Range: 0 - 40 °C
pH Range: 1 – 14
Compatible with a number of solvents. (Not compatible with ethyl acetate.)

IV. Dimensions

Overall Length (fully assembled with cap): 4.5 cm

V. Capacities

Maximum Sample Volume: 500 µl
Final Concentrate Volume: 15-20 µl
Filtrate Receiver Volume: 500 µl
Hold-up Volume (membrane/support): <5 µl

VI. Centrifuge

Fits rotors that accept 1.5 ml tubes
Maximum Centrifugal Force: 14.000 x g

VII. Choosing the appropriate MWCO

For convenient and successful application, centrifugal devices with membranes in 4 colour-coded molecular weight cut-offs (MWCO) are available. In order to choose the appropriate MWCO please use the following thumb-rule:

Centrifugation of proteins/peptides:
MWCO ≤ 1/3 of sample molecule size
Centrifugation of nucleic acids:
MWCO ≤ 1/2 sample molecule size

MWCO	Size of Biomolecules	Molecular Weight of Biomolecules
3 kD		10 – 20 kD
10 kD		30 – 90 kD
30 kD		90 – 180 kD
100 kD	30 – 90 nm	> 300 kD

For ultra filtration of DNA samples please note:
During ultrafiltration, solubilised DNA behaves differently than globular protein molecules. We recommend to use the following MWCOs:

Molecular Size (bases or bp)	MWCO
< 50 bp	3 kD
50 – 200 bp	10 kD
200 bp – 1 kb	30 kD
> 1 kb	100 kD

II. Purity

VIII.1. Sanitisation

Provided non-sterile; may be sanitised by filtering 70 % ethanol through the device prior to use.

1. Fill the sample reservoir with 70 % ethanol. Close the cap and centrifuge at 14.000 x g until all liquid passes through the membrane.
2. Discard the filtrate. Remove residual ethanol by filling the device with sterile water and centrifuge again.

Use the device within 20 minutes to prevent membrane dehydration.

VIII.2. Pre-Rinsing (optional)

Due to manufacturing processes, the membrane contains trace amounts of glycerol and sodium azide. If these reagents are likely to interfere with a subsequent assay, they may be removed by filtering 500 µl of deionised water or buffer through the membrane two times. Additionally, as a first step 500 µl of a 50 mM NaOH solution may be filtered by centrifugation through the membrane. Then wash twice with 500 µl deionised water by centrifugation at 14.000 x g for 5 minutes.
Please note: Make sure that the membrane does not dry until you load the sample!

IX. Operation

The MINI filtration device has a very fast flow rate. Typically, 4 to 20 min. are sufficient to concentrate a sample.

1. Be sure that the sample reservoir is firmly placed into the filtrate receiver.
2. Pipette 50 – 500 µl of the sample into the sample reservoir. Cap the device.
3. Place the filtration device into a fixed-angle centrifuge rotor that accepts 1.5 ml tubes. Always counterbalance the rotor with another device containing an equivalent sample volume.
4. Spin up to 14.000 x g for the required length of time (approx. 4 – 20 min.) to achieve the desired concentrate volume. For DNA retention, do not exceed 5.000 x g.
5. Stop the centrifuge and remove the filtrate receiver. Concentrated sample is recovered with a micropipette from the sample reservoir.
   Note: It may appear as if the sample has “spun dry” in the filtration device. However, the sample may easily be recovered by pipetting approx. 20 µl of water or buffer onto the membrane.

Carl Roth GmbH + Co. KG

Schoemperlenstraße 3-5 • 76185 Karlsruhe
P.O. Box 100121 • 76231 Karlsruhe
Phone: +49 (0) 721/ 5606-0
Fax: +49 (0) 721/ 5606-149
info@carlroth.com • www.carlroth.com
ip 07/2021

Notes regarding specific applications:

Preparation of protein samples for electrophoresis can easily be done with the ROTI®Spin centrifugal devices. ROTI®Load loading buffer may be added directly to the concentrate cup for heating in the water bath.
For salt removal or buffer exchange concentrate sample 10 fold. Then dilute again 10fold and repeat the concentration. Repeat 3-5 times to remove 95 – 99 % of salts or buffer.

X. Trouble shooting

Problem	
Possible Cause	*Recommended Solution*
Spin time needed for concentration is too long	
Centrifuge speed to low	Increase speed, re-calibrate centrifuge
Gel layer built up	Check rotors, use fixed angle rotor
Biomolecule reached maximum concentration	Spread solution to more than 1 filtration device
Loss of sample activity in subsequent assay	
Interference with glycerol or sodium azide	Pre-rinse filtration devices prior to use (see VII.2.)
Incompatibility of biomolecule and solvent	Adjust pH, change buffer
Protein:protein interactions, gel layer formation	Increase final concentration volume
Loss of biomolecules / recovery rate too low	
Biomolecules are not retained by the membrane	Select lower MWCO
Sub-units of biomolecule pass through the membrane	Select lower MWCO
Proteins stick to the plastic material	Pretreat with glycerol (see XI)
g-force too high	Reduce speed to 5.000 x g or less
Presence of unwanted molecules in the sample	
Wrong MWCO	Select higher MWCO

XI. Pretreatment

The material used for construction of ROTI®Spin MINI centrifuge devices has been advanced to reduce the plastic adherence of proteins. However, some percentage of “sticky” proteins may still adhere to the devices. In these cases, the following pretreatment of the centrifuge device is recommended:

1. Fill reservoir with 500 µl of a 10% glycerol solution.
2. Soak overnight at room temperature.
3. Discard the glycerol and rinse with deionised water without centrifugation.
4. Fill with 500 µl deionised water and centrifuge at 14.000 x g for 5 minutes.
5. Repeat step 4.

Please note: Make sure that the membrane does not dry until you load the sample!

ROTI®Spin MINI-3	**25 units**	**CL12.1**
ROTI®Spin MINI-10	**25 units**	**CL13.1**
ROTI®Spin MINI-30	**25 units**	**CL14.1**
ROTI®Spin MINI-100	**25 units**	**CL15.1**