



BIOKÉ
sharing knowledge

Plesmanlaan 1d
2333 BZ Leiden
The Netherlands
T. +31 (0)71 568 10 00
T. Belgium: 0800 71640
F. +31 (0)71 568 10 10
info@bioke.com
www.bioke.com

Small RNA and DNA from plasma

User manual

NucleoSpin® miRNA Plasma

May 2011 / Rev. 01





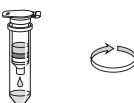


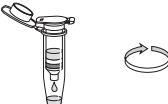
MACHEREY-NAGEL



Isolation of miRNA from plasma

Protocol-at-a-glance (Rev.01)

NucleoSpin® miRNA Plasma

1	Prepare sample		300 µL plasma or serum* 90 µL MLP Vortex 5 s RT, 3 min
2	Precipitate protein		30 µL MPP Vortex 5 s RT, 1 min 11,000 x g, 3 min
3	Transfer supernatant		Transfer clear supernatant to Collection Tube (2 mL, lid)
4	Adjust binding conditions		400 µL isopropanol Vortex 5 s
5	Bind RNA and DNA		Load sample on NucleoSpin® miRNA Column RT, 2 min 11,000 x g, 30 s
6	Optional: Digest DNA		Optional: 1st 700 µL MW2 11,000 x g, 30 s 2nd 250 µL MW2 11,000 x g, 2 min 50 µL rDNase in Reaction Buffer for rDNase RT, 15 min
7	Wash and dry		1st 100 µL MW1 11,000 x g, 30 s 2nd 700 µL MW2 11,000 x g, 30 s 3rd 250 µL MW2 11,000 x g, 2 min
8	Elute RNA		30 µL RNase-free H ₂ O RT, 1 min 11,000 x g, 1 min

* Larger sample volumes can be processed when buffer volumes of MLP, MPP, and isopropanol are increased proportionally. Multiple loading is necessary in step 5 (see section 2.3 for more information).

Table of contents

1	Components	4
1.1	Kit contents	4
1.2	Reagents, consumables, and equipment to be supplied by user	5
1.3	About this user manual	5
2	Product description	6
2.1	The basic principle	6
2.2	Kit specifications	6
2.3	Amount of starting material	7
2.4	Proteinase K digest	8
2.5	Addition of carrier	8
2.6	Elution procedures	8
3	Storage conditions and preparation of working solutions	9
4	Safety instructions – risk and safety phrases	10
4.1	Risk and safety phrases	10
4.2	GHS classification	11
5	Protocol – small RNA and DNA purification from plasma or serum	13
6	Appendix	17
6.1	Troubleshooting	17
6.2	Ordering information	18
6.3	Product use restriction/warranty	19

1 Components

1.1 Kit contents

NucleoSpin® miRNA Plasma			
REF	10 preps 740981.10	50 preps 740981.50	250 preps 740981.250
Lysis Buffer MLP	3 mL	15 mL	75 mL
Protein Precipitation Buffer MPP	5 mL	5 mL	30 mL
Reaction Buffer for rDNase	3 mL	3 mL	30 mL
rDNase, RNase-free (lyophilized)	1 vial (size C)	1 vial (size C)	5 vials (size C)
Wash Buffer MW1	10 mL	10 mL	35 mL
Wash Buffer MW2 (Concentrate)*	6 mL	20 mL	2 x 40 mL
RNase-free H ₂ O	5 mL	5 mL	15 mL
NucleoSpin® miRNA Columns (green rings)	10	50	250
Collection Tubes (1.5 mL)	10	50	250
Collection Tubes (2 mL)	10	50	250
Collection Tubes (2 mL, lid)	10	50	250
User manual	1	1	1

* For preparation of working solutions and storage conditions see section 3.

1.2 Reagents, consumables, and equipment to be supplied by user

Reagents

- 96–100 % ethanol
- Isopropanol

Consumables

- RNase-free disposable pipette tips

Equipment

- Manual pipettors
- Vortexer
- Centrifuge for microcentrifuge tubes
- Personal protection equipment (lab coat, gloves, goggles)

1.3 About this user manual

It is strongly recommended that first-time users of the **NucleoSpin® miRNA Plasma** kit read the detailed protocol section of this user manual. Experienced users, however, may refer to the Protocol-at-a-glance instead. The Protocol-at-a-glance is designed to be used only as a supplemental tool for quick referencing while performing the purification procedure.

All technical literature is available on the internet at ***www.mn-net.com***.

2 Product description

2.1 The basic principle

The **NucleoSpin® miRNA Plasma** kit offers the unique feature to isolate small RNA and DNA from plasma without the need to resort to the cumbersome phenol / chloroform extraction or a time consuming proteinase digest.

The sample material is denatured in Lysis Buffer MLP. The protein is then precipitated by Protein Precipitation Buffer MPP and pelleted by centrifugation.

After the removal of protein the binding conditions for nucleic acids are adjusted by adding isopropanol.

The small RNA and DNA are bound to the NucleoSpin® miRNA Column. Optionally, DNA can be removed by an on-column rDNase digest. The remaining nucleic acids are washed and eluted with minimal amounts of RNase-free water.

2.2 Kit specifications

- The **NucleoSpin® miRNA Plasma** kit is designed for the isolation of short RNA and DNA (< 1000 nt/bp) from cell free blood plasma or serum.
- **rDNase** is provided for an optional on-column digest to remove traces of genomic DNA.
- The eluted RNA and DNA are ready-to-use for all standard downstream applications, for example, qPCR, qRT-PCR, Northern Blot, chip hybridization.

Table 1: Kit specifications at a glance

Parameter	NucleoSpin® miRNA Plasma
Sample size	300 µL blood plasma or serum
Fragment size	< 1000 nt/bp
Binding capacity	200 µg
Elution volume	20–50 µL
Preparation time	40 min/10 preps (without rDNase digest) 70 min/10 preps (with rDNase digest)

2.3 Amount of starting material

The standard procedure allows to process 300 μL of sample material with only one loading step onto the NucleoSpin[®] miRNA Column. This is usually enough to detect also low abundance miRNA in plasma or serum.

If larger sample volumes are to be used to increase the sensitivity even further, the volumes of Buffer MLP and Buffer MPP as well as the isopropanol have to be increased proportionally. Multiple loading steps per sample are necessary.

But, consider that doubling or tripling the starting volume will result in an only 1–1.5 cycles earlier signal in qRT-PCR, which is rather insignificant for detection sensitivity compared to the much larger differences that occur from sample to sample or between different miRNAs. Furthermore, if plasma quality is low, co-purified RT-PCR inhibitors might require diluting the eluate and thereby counteract the increased yields.

Figure 1 shows qRT-PCR results from eight different plasma samples analyzed for miR-16 (very high expression) and miR-1 (very low expression). They differ in average by about 14 cycles, which is a difference in expression by 3–4 orders of magnitude (1.000–10.000 fold)! Doubling the amount of sample material would just shift the mean values from 32.6 to 31.6 (miR-1) and from 18.3 to 17.3 (miR-16). This is much less than the variation from sample to sample and can thus be neglected.

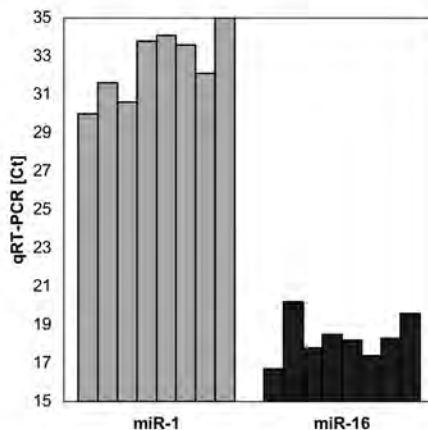


Figure 1: qRT-PCR quantification of miR-1 and miR-16

MiRNA from only 300 μL of eight different blood plasma samples were purified and eluted in 30 μL RNase-free water. 2 μL of each eluate were used for a 10 μL RT reaction (Applied Biosystems, TaqMan[®] MicroRNA RT Kit) with miRNA specific primers (Applied Biosystems, hsa-miR-1 MicroRNA Assay and hsa-miR-16 MicroRNA Assay). The RT reaction mix was diluted 1:10. Only 4 μL of the resulting 100 μL were used for the PCR reaction (Applied Biosystems TaqMan[®] Universal PCR Master Mix) in combination with the MicroRNA Assays for specific priming mentioned before.

2.4 Proteinase K digest

A short protein digestion step might increase miRNA yield, especially for low quality, hemolyzed plasma. Add 10 μL of Proteinase K ($\sim 30 \mu\text{g}/\mu\text{L}$ in Buffer PB, see ordering information) to 300 μL plasma, incubate for 10 min at 37 °C, and then proceed with addition of Buffer MLP according to the protocol.

2.5 Addition of carrier

To improve RNA/DNA binding to the NucleoSpin® miRNA Column, carrier can be added to the sample after the removal of precipitated protein. Slightly higher yields could be found with 2 μg of glycogen or 5 μg of LPA (linear polyacrylamide). However, negligible effects were observed for Poly-A which furthermore interferes with a photometric quantification of the purified nucleic acids.

2.6 Elution procedures

The elution buffer volume does not only influence total yield and concentration of RNA and DNA, but does also influence the ratio between very small and larger oligonucleotides:

20 μL

The silica membrane is not completely wetted. Only weakly binding very small oligonucleotides like miRNA are eluted efficiently. Larger RNA and DNA are more likely to remain bound to the column. The eluted miRNA is highly concentrated.

30 μL (standard)

The standard elution buffer volume of 30 μL is sufficient to wet the silica membrane completely. It results a high total yield of miRNA/RNA/DNA and simultaneously maximizes the concentration.

50 μL

Increasing the elution buffer volume will further increase the final yield but consequently will reduce the concentration. The gain in yield will usually not compensate for the loss in sensitivity of miRNA detection caused by the dilution of the eluate. Furthermore, larger RNA and DNA will be eluted more efficiently, which might, however, be interesting for the analysis of circulating DNA.

3 Storage conditions and preparation of working solutions

Attention:

Buffers MLP and MW1 contain guanidinium thiocyanate. Wear gloves and goggles!

Storage conditions:

- Store lyophilized RNase-free rDNase at +4 °C on arrival (stable for at least one year).
- All other kit components should be stored at room temperature (18–25 °C) and are stable for at least one year. Storage at lower temperatures may cause precipitation of salts.

Before starting the first **NucleoSpin® miRNA Plasma** procedure prepare the following:

- **Wash Buffer MW2:** Add the indicated volumes of 96–100 % ethanol to the MW2 concentrate. The buffer can be stored at room temperature (18–25 °C) for at least one year.
- **RNase-free rDNase:** Add the indicated volume of Reaction Buffer for rDNase to the rDNase vial and incubate for 1 min at room temperature. Gently swirl the vial to completely dissolve the rDNase. Be careful not to mix rDNase vigorously as rDNase is sensitive to mechanical agitation. Dispense into aliquots and store at -20 °C. The frozen solution is stable for at least 6 months. Do not freeze / thaw the aliquots more than three times.




NucleoSpin® miRNA Plasma			
REF	10 preps 740981.10	50 preps 740981.50	250 preps 740981.250
Wash Buffer MW2 (Concentrate)	6 mL Add 24 mL ethanol	20 mL Add 80 mL ethanol	2 x 40 mL Add 160 mL ethanol to each bottle
RNase-free rDNase (lyophilized)	1 vial (size C) Add 3 mL Reaction Buffer for rDNase	1 vial (size C) Add 3 mL Reaction Buffer for rDNase	5 vials (size C) Add 3 mL Reaction Buffer for rDNase to each vial

4 Safety instructions – risk and safety phrases

The following components of the **NucleoSpin® miRNA Plasma** kits contain hazardous contents.

Wear gloves and goggles and follow the safety instructions given in this section.

4.1 Risk and safety phrases

Component	Hazard contents	Hazard symbol	Risk phrases	Safety phrases
Inhalt	Gefahrstoff	Gefahrstoff-symbol	R-Sätze	S-Sätze
rDNase, RNase-free	rDNase, lyophilized <i>rDNase (lyophilisiert)</i>	 Xn	R 42/43	S 22–24
MLP	Guanidinium thiocyanate <i>Guanidiniumthiocyanat</i>	 Xn*	R 20/21/22-32-52/53	S 13-61
MW1	Guanidinium thiocyanate + ethanol <i>Guanidiniumthiocyanat + Ethanol</i>	 F**	R 11	S 7-16

Risk phrases / R-Sätze

R 11	Highly Flammable. <i>Leichtentzündlich.</i>
R 20/21/22	Harmful by inhalation, in contact with the skin and if swallowed. <i>Gesundheitsschädlich beim Einatmen, Verschlucken und Berührung mit der Haut.</i>
R 32	Contact with acids liberates very toxic gas. <i>Entwickelt bei Berührung mit Säure sehr giftige Gase.</i>
R 42/43	May cause sensitization by inhalation and skin contact. <i>Sensibilisierung durch Einatmen und Hautkontakt möglich.</i>
R 52/53	Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment. <i>Giftig für Wasserorganismen, kann in Gewässern längerfristig schädliche Wirkungen haben.</i>

Safety phrases / S-Sätze

S 7	Keep container tightly closed. <i>Behälter dicht geschlossen halten.</i>
-----	---

* Hazard labeling not necessary if quantity per bottle below 125 g or mL (certificate of exemption according to 67/548/EEC Art. 25, 1999/45/EC Art. 12 and German GefStoffV § 20 (3) and TRGS 200 7.1). For further information see Material Safety Data Sheet.




**Hazard labeling not necessary if quantity per bottle below 25 g or mL (certificate of exemption according to 67/548/EEC Art. 25, 1999/45/EC Art. 12 and German GefStoffV § 20 (3) and TRGS 200 7.1). For further information see Material Safety Data Sheet.

S 13	Keep away from food, drink and animal feedstuffs. <i>Von Nahrungsmitteln, Getränken und Futtermitteln fernhalten.</i>
S 16	Keep away from sources of ignition – No smoking. <i>Von Zündquellen fernhalten – Nicht rauchen.</i>
S 22	Do not breathe dust. <i>Staub nicht einatmen.</i>
S 24	Avoid contact with skin. <i>Berührung mit der Haut vermeiden.</i>
S 61	Avoid release to the environment. Refer to special instructions / safety data sheets. <i>Freisetzung in die Umwelt vermeiden. Besondere Anweisungen einholen/Sicherheitsdatenblatt zu Rate ziehen.</i>

4.2 GHS classification

Only harmful features need not be labeled with H and P phrases until 125 mL or 125 g.

Mindergefährliche Eigenschaften müssen bis 125 mL oder 125 g nicht mit H- und P-Sätzen gekennzeichnet werden.

Component	Hazard contents	GHS symbol	Hazard phrases	Precaution phrases
Inhalt	Gefahrstoff	GHS Symbol	H-Sätze	P-Sätze
rDNase, RNase-free	rDNase, lyophilized <i>rDNase (lyophilisiert)</i>	 Warning <i>Achtung</i>	317-334	261-304+341-342+311-301+312-280-302+352-333+313
MLP	Guanidinium thiocyanate 30–60% <i>Guanidiniumthiocyanat 30–60%</i>	 Warning <i>Achtung</i>	302-412-EUH031	260-273-301+312-330
MW1	Guanidinium thiocyanate 1–15% + ethanol 55–75% <i>Guanidiniumthiocyanat 1–15% + Ethanol 55–75%</i>	 Danger <i>Gefahr</i>	225	210-233-403+235

Hazard phrases

H 225	Highly flammable liquid and vapour. <i>Flüssigkeit und Dampf leicht entzündbar.</i>
H 302	Harmful if swallowed. <i>Gesundheitsschädlich bei Verschlucken.</i>
H 317	May cause an allergic skin reaction. <i>Kann allergische Hautreaktionen verursachen.</i>
H 334	May cause allergy or asthma symptoms or breathing difficulties if inhaled.

Kann bei Einatmen Allergie, asthmaartige Symptome oder Atembeschwerden verursachen.

H 412 Harmful to aquatic life with long lasting effects.
Schädlich für Wasserorganismen, mit langfristiger Wirkung.

EUH 031 Contact with acids liberates toxic gas.
Entwickelt bei Berührung mit Säure giftige Gase.

Precaution phrases

P 210 Keep away from heat / sparks / open flames / hot surfaces. – No smoking.
Von Hitze / Funken / offener Flamme / heißen Oberflächen fernhalten. – Nicht rauchen.

P 233 Keep container tightly closed.
Behälter dicht verschlossen halten.

P 260 Do not breathe dust / fume / gas / mist / vapours / spray.
Staub / Rauch / Gas / Nebel / Dampf / Aerosol nicht einatmen.

P 261 Avoid breathing dust.
Einatmen von Staub vermeiden.

P 273 Avoid release to the environment.
Freisetzung in die Umwelt vermeiden.

P 280 Wear protective gloves / eye protection.
Schutzhandschuhe / Augenschutz tragen.

P 301+312 IF SWALLOWED: Call a POISON CENTER or doctor / physician if you feel unwell.
Bei Verschlucken: Bei Unwohlsein Giftinformationszentrum oder Arzt anrufen.

P 302+352 IF ON SKIN: Wash with plenty of soap and water.
Bei Kontakt mit der Haut: Mit viel Wasser und Seife waschen.

P 304+341 IF INHALED: If breathing is difficult, remove to fresh air and keep at rest in a position comfortable for breathing.
Bei Einatmen: Bei Atembeschwerden an die frische Luft bringen und in einer Position ruhigstellen, die das Atmen erleichtert.

P 330 Rinse mouth.
Mund ausspülen.

P 333+313 If skin irritation occurs: Get medical advice / attention.
Bei Hautreizung: Ärztlichen Rat einholen / ärztliche Hilfe hinzuziehen.

P 342+311 If experiencing respiratory symptoms: Call a POISON CENTER or doctor / physician.
Bei Symptomen der Atemwege: Giftinformationszentrum oder Arzt anrufen.

P 403+235 Store in a well-ventilated place. Keep cool.
An einem gut belüfteten Ort lagern. Kühl halten.

For further information please see Material Safety Data Sheets (www.mn-net.com).
Weiterführende Informationen finden Sie in den Sicherheitsdatenblättern (www.mn-net.com).

5 Protocol – small RNA and DNA purification from plasma or serum

Before starting with the preparation check that isopropanol is available, that ethanol was added to Wash Buffer MW2, and that rDNase was reconstituted according to section 3.

1 Prepare sample

Note: See section 2.4 for optional Proteinase K digest.

Add **90 µL Buffer MLP** to **300 µL sample**.

Vortex for **5 s**.

Incubate for **3 min** at **room temperature (18–25 °C)**.

Note: To process 600 µL or 900 µL sample material, increase volumes for Buffer MLP, MPP, and isopropanol proportionally. Multiple loading steps will be necessary in step 5. See section 2.3 for more information.



+ 90 µL MLP

Vortex 5 s

**RT
3 min**

2 Precipitate protein

Add **30 µL Buffer MPP** and vortex for **5 s**.

Incubate for **1 min** at **room temperature (18–25 °C)**.

Centrifuge for **3 min** at **11,000 x g** to pellet the protein.



+ 30 µL MPP

Vortex 5 s

**RT
1 min**

**11,000 x g
3 min**



3 Transfer supernatant

Transfer the clear supernatant into a new Collection Tube (2 mL, lid).



**Transfer
supernatant**

4 Adjust binding conditions

Note: Addition of carrier, for example, 2 µg of glycogen or 5 µg of LPA (linear polyacrylamide), might slightly improve the miRNA yield but usually is not necessary. Poly-A has shown only negligible effects and furthermore might interfere with photometric miRNA quantification.

Add **400 µL isopropanol** and vortex for **5 s**.



**+ 400 µL
isopropanol**

Vortex 5 s

5 Bind RNA and DNA

Place a **NucleoSpin® miRNA Column** in a Collection Tube (2 mL) and load the sample onto the column.

Incubate for **2 min** at **room temperature (18–25 °C)**.

Centrifuge for **30 s** at **11,000 x g**.

Discard the flow-through and place the column back into the collection tube.

If more than 300 µL plasma / serum was used, repeat this step until all sample is loaded onto the column.



Load sample

RT
2 min

11,000 x g
30 s



Repeat step
if necessary

6 Optional: DNA digest

***Note:** Co-purified DNA might interfere with qPCR quantification of miRNA. The following on-column digest degrades bound DNA including miRNA genes.*

However, if miRNA specific qPCR detection systems are used or cell free plasma DNA is needed intact for further analysis, skip the rDNase digest and proceed directly with step 7.

1st wash

Add **700 µL Buffer MW2** to the NucleoSpin® miRNA Column. Centrifuge for **30 s** at **11,000 x g**. Discard flow-through and place the column back into the collection tube.



+ 700 µL MW2

11,000 x g
30 s



2nd wash

Add **250 µL Buffer MW2** to the NucleoSpin® miRNA Column. Centrifuge for **2 min** at **11,000 x g**. It is not necessary to discard the flow-through.



+ 250 µL MW2

11,000 x g
2 min



Digest DNA

Add **50 µL rDNase** (dissolved in Reaction Buffer for rDNase according to section 3) directly onto the silica membrane of the NucleoSpin® miRNA Column.

Close the lid and incubate at **room temperature (18–25 °C)** for **15 min**.



**+ 50 µL
rDNase**

**RT
15 min**

7 Wash and dry silica membrane

1st wash

Add **100 µL Buffer MW1** to the NucleoSpin® miRNA Column. Centrifuge for **30 s** at **11,000 x g**.

Discard flow-through and place the column back into the collection tube.



+ 100 µL MW1



**11,000 x g
30 s**

2nd wash

Add **700 µL Buffer MW2** to the NucleoSpin® miRNA Column. Centrifuge for **30 s** at **11,000 x g**.

Discard flow-through and place the column back into the collection tube.



+ 700 µL MW2



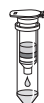
**11,000 x g
30 s**

3rd wash

Add **250 µL Buffer MW2** to the NucleoSpin® miRNA Column.

Centrifuge for **2 min** at **11,000 x g** to dry the membrane completely.

If the liquid in the collection tube has touched the NucleoSpin® miRNA Column after the 3rd wash, discard flow-through and centrifuge again.



+ 250 µL MW2



**11,000 x g
2 min**

Note: The same collection tube is used throughout the entire washing procedure to reduce plastic waste. If new collection tubes are to be used for each step, see ordering information.

8 Elute RNA and DNA

Place the NucleoSpin® miRNA Column in a new Collection Tube (1.5 mL).

Note: The elution buffer volume highly influences the final yield and concentration and, furthermore, influences elution efficiency of large oligonucleotides. See section 2.6 for more information about elution in 20, 30 or 50 µL.

Add **30 µL RNase-free H₂O** directly onto the silica membrane of the column.

Incubate for **1 min** at **room temperature (18–25 °C)**.

Close the lid and centrifuge for **1 min** at **11,000 x g**.



**+ 30 µL
RNase-free
H₂O**

**RT
1 min**



**11,000 x g
1 min**

6 Appendix

6.1 Troubleshooting

Problem	Possible cause and suggestions
Poor or no RNA yield	<i>Reagents not applied or restored properly</i>
	<ul style="list-style-type: none">• Always dispense exactly the buffer volumes given in the protocols! The correct proportions of buffers MLP, MPP, and isopropanol are essential for optimal yield and purity.
	<ul style="list-style-type: none">• Always follow closely the given instructions with regard to order and mode of mixing (shaking, vortexing etc).
	<ul style="list-style-type: none">• Add the indicated volume of 96–100 % ethanol to Buffer MW2 Concentrate and mix thoroughly.
	<ul style="list-style-type: none">• Store kit components at room temperature (18–25 °C). Storage at lower temperatures may cause salt precipitation. Heat buffer with precipitated salt to 30 °C until salt is dissolved. Let the buffer cool down to room temperature before use.
RNA is degraded	<ul style="list-style-type: none">• Keep bottles tightly closed in order to prevent evaporation or contamination.
	<i>RNase contamination</i>
Suboptimal performance of RNA in downstream experiments	<ul style="list-style-type: none">• Create an RNase-free working environment. Wear gloves during all steps of the procedure. Change gloves frequently. Use sterile, disposable polypropylene tubes and filter tips. Keep tubes closed whenever possible during the preparation unless stated otherwise. Glassware should be oven-baked for at least 2 hours at 250 °C before use.
	<i>Inhibition by co-purified RT-PCR inhibitors</i>
	<ul style="list-style-type: none">• Heme, hemin, and other degradation products of red blood cells strongly inhibit reverse transcription and PCR. Too much plasma or bad plasma quality can result in contamination with these inhibitors. Use <u>less</u> plasma, dilute eluates, perform the optional Proteinase K digest (see section 2.4), or add BSA prior to RT or PCR reactions.

Problem	Possible cause and suggestions
Suboptimal performance of RNA in downstream experiments (continued)	<i>Carry-over of ethanol or salt</i> <ul style="list-style-type: none">Do not let the flow-through touch the column outlet after the second MW2 wash. Make sure to centrifuge at the corresponding speed for the respective time in order to remove ethanolic buffer MW2 completely.Check if buffer MW2 has been equilibrated to room temperature (18–25 °C) before use. Washing at lower temperatures lowers efficiency of salt removal.
	<i>Store isolated RNA properly</i> <ul style="list-style-type: none">Eluted RNA should always be kept on ice for optimal stability since trace contaminations of omnipresent RNases (general lab ware, fingerprints, dust) will degrade the isolated RNA. For short term storage freeze at -20 °C, for long term storage freeze at -70 °C.

6.2 Ordering information

Product	REF	Pack of
NucleoSpin® miRNA Plasma	740981.10/.50/.250	10/50/250 preps
NucleoSpin® miRNA	740971.10/.50/.250	10/50/250 preps
rDNase Set (1 vial rDNase (size D), 7 mL Reaction Buffer for rDNase)	740963	1 set
Proteinase K	740506	100 mg
Collection Tubes (2 mL)	740600	1000

Visit **www.mn-net.com** for more detailed product information.

6.3 Product use restriction/warranty

NucleoSpin® miRNA Plasma kit components are intended, developed, designed, and sold FOR RESEARCH PURPOSES ONLY, except, however, any other function of the product being expressly described in original MACHEREY-NAGEL product leaflets.

MACHEREY-NAGEL products are intended for GENERAL LABORATORY USE ONLY! MACHEREY-NAGEL products are suited for QUALIFIED PERSONNEL ONLY! MACHEREY-NAGEL products shall in any event only be used wearing adequate PROTECTIVE CLOTHING. For detailed information please refer to the respective Material Safety Data Sheet of the product! MACHEREY-NAGEL products shall exclusively be used in an ADEQUATE TEST ENVIRONMENT. MACHEREY-NAGEL does not assume any responsibility for damages due to improper application of our products in other fields of application. Application on the human body is STRICTLY FORBIDDEN. The respective user is liable for any and all damages resulting from such application.

DNA/RNA/PROTEIN purification products of MACHEREY-NAGEL are suitable for *IN-VITRO*-USES ONLY!

ONLY MACHEREY-NAGEL products specially labeled as IVD are also suitable for *IN-VITRO*-diagnostic use. Please pay attention to the package of the product. *IN-VITRO*-diagnostic products are expressly marked as IVD on the packaging.

IF THERE IS NO IVD SIGN, THE PRODUCT SHALL NOT BE SUITABLE FOR *IN-VITRO*-DIAGNOSTIC USE!

ALL OTHER PRODUCTS NOT LABELED AS IVD ARE NOT SUITED FOR ANY CLINICAL USE (INCLUDING, BUT NOT LIMITED TO DIAGNOSTIC, THERAPEUTIC AND/OR PROGNOSTIC USE).

No claim or representations is intended for its use to identify any specific organism or for clinical use (included, but not limited to diagnostic, prognostic, therapeutic, or blood banking). It is rather in the responsibility of the user or - in any case of resale of the products - in the responsibility of the reseller to inspect and assure the use of the DNA/RNA/protein purification products of MACHEREY-NAGEL for a well-defined and specific application.

MACHEREY-NAGEL shall only be responsible for the product specifications and the performance range of MN products according to the specifications of in-house quality control, product documentation and marketing material.

This MACHEREY-NAGEL product is shipped with documentation stating specifications and other technical information. MACHEREY-NAGEL warrants to meet the stated specifications. MACHEREY-NAGEL's sole obligation and the customer's sole remedy is limited to replacement of products free of charge in the event products fail to perform as warranted. Supplementary reference is made to the general business terms and conditions of MACHEREY-NAGEL, which are printed on the price list. Please contact us if you wish to get an extra copy.

There is no warranty for and MACHEREY-NAGEL is not liable for damages or defects arising in shipping and handling (transport insurance for customers excluded), or out of accident or improper or abnormal use of this product; defects in products or

components not manufactured by MACHEREY-NAGEL, or damages resulting from such non-MACHEREY-NAGEL components or products.

MACHEREY-NAGEL makes no other warranty of any kind whatsoever, and SPECIFICALLY DISCLAIMS AND EXCLUDES ALL OTHER WARRANTIES OF ANY KIND OR NATURE WHATSOEVER, DIRECTLY OR INDIRECTLY, EXPRESS OR IMPLIED, INCLUDING, WITHOUT LIMITATION, AS TO THE SUITABILITY, REPRODUCTIVITY, DURABILITY, FITNESS FOR A PARTICULAR PURPOSE OR USE, MERCHANTABILITY, CONDITION, OR ANY OTHER MATTER WITH RESPECT TO MACHEREY-NAGEL PRODUCTS.

In no event shall MACHEREY-NAGEL be liable for claims for any other damages, whether direct, indirect, incidental, compensatory, foreseeable, consequential, or special (including but not limited to loss of use, revenue or profit), whether based upon warranty, contract, tort (including negligence) or strict liability arising in connection with the sale or the failure of MACHEREY-NAGEL products to perform in accordance with the stated specifications. This warranty is exclusive and MACHEREY-NAGEL makes no other warranty expressed or implied.

The warranty provided herein and the data, specifications and descriptions of this MACHEREY-NAGEL product appearing in MACHEREY-NAGEL published catalogues and product literature are MACHEREY-NAGEL's sole representations concerning the product and warranty. No other statements or representations, written or oral, by MACHEREY-NAGEL's employees, agent or representatives, except written statements signed by a duly authorized officer of MACHEREY-NAGEL are authorized; they should not be relied upon by the customer and are not a part of the contract of sale or of this warranty.

Product claims are subject to change. Therefore please contact our Technical Service Team for the most up-to-date information on MACHEREY-NAGEL products. You may also contact your local distributor for general scientific information. Applications mentioned in MACHEREY-NAGEL literature are provided for informational purposes only. MACHEREY-NAGEL does not warrant that all applications have been tested in MACHEREY-NAGEL laboratories using MACHEREY-NAGEL products. MACHEREY-NAGEL does not warrant the correctness of any of those applications.

Last updated: 07/2010, Rev. 03

Please contact:
MACHEREY-NAGEL GmbH & Co. KG
Tel.: +49 24 21 969-270
tech-bio@mn-net.com

Trademarks/Disclaimer:

NucleoSpin® is a registered trademark of MACHEREY-NAGEL GmbH & Co. KG
TaqMan® is a registered trademark of Roche Molecular Systems, Inc.

All used names and denotations can be brands, trademarks, or registered labels of their respective owner – also if they are not special denotation. To mention products and brands is only a kind of information (i.e., it does not offend against trademarks and brands and can not be seen as a kind of recommendation or assessment). Regarding these products or services we can not grant any guarantees regarding selection, efficiency, or operation.