



Genomic DNA from organs and cells

User manual

NucleoSpin[®] DNA RapidLyse

May 2018 / Rev. 03

Genomic DNA from organs and cells

Protocol at a glance (Rev. 03)

NucleoSpin® DNA RapidLyse

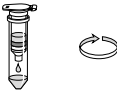
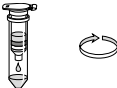
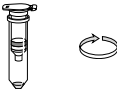
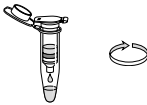
1 Lyse sample		Up to 40 mg wet weight sample or 1 x 10 ⁶ cells in a 2 mL tube 150 µL RLY 10 µL Liquid Proteinase K 56 °C, 1 h, thermomixer at maximum speed						
2 Adjust DNA binding conditions		440 µL RLB Vortex 5 s						
3 Bind DNA		Load 640 µL lysate on a NucleoSpin® DNA RapidLyse Column 11,000 x g, 1 min						
4 Wash silica membrane		<table><tr><td>1st</td><td>500 µL RLW</td><td>11,000 x g, 1 min</td></tr><tr><td>2nd</td><td>500 µL RLW</td><td>11,000 x g, 1 min</td></tr></table>	1st	500 µL RLW	11,000 x g, 1 min	2nd	500 µL RLW	11,000 x g, 1 min
1st	500 µL RLW	11,000 x g, 1 min						
2nd	500 µL RLW	11,000 x g, 1 min						
5 Dry silica membrane		11,000 x g, 1 min						
6 Elute DNA		100 µL RLE 11,000 x g, 1 min						

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1 Components

1.1 Kit contents

NucleoSpin® DNA RapidLyse			
REF	10 preps 740100.10	50 preps 740100.50	250 preps 740100.250
Lysis Buffer RLY	13 mL	13 mL	60 mL
Binding Buffer RLB	25 mL	25 mL	125 mL
Wash Buffer RLW (Concentrate)*	6 mL	12 mL	3 x 25 mL
Elution Buffer RLE**	13 mL	13 mL	30 mL
Liquid Proteinase K	120 µL	600 µL	2 x 1.5 mL
NucleoSpin® DNA RapidLyse Columns (light green rings)	10	50	250
Collection Tubes (2 mL)	20	100	500
User manual	1	1	1

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

**Composition of Elution Buffer RLE: 5 mM Tris/HCl, pH 8.5

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

Reagents

- 96–100 % ethanol (for preparation of Wash Buffer RLW)

Consumables

- 2 mL microcentrifuge tubes for sample lysis
- 1.5 mL microcentrifuge tubes for DNA elution
- Disposable pipette tips

Equipment

- Manual pipettors
- Centrifuge for microcentrifuge tubes
- Vortex mixer (e.g., Vortex-Genie® 2 from Scientific Industries)
- Thermomixer (e.g., ThermoMixer® C from Eppendorf for 2 mL tubes)
- Personal protection equipment (lab coat, gloves, goggles)
- For challenging samples (protocol 5.2): MN Bead Tube Holder and Bead Tubes Type F

1.3 About this user manual

It is strongly recommended for first time users to read the detailed protocol sections of the **NucleoSpin® DNA RapidLyse** kit before using this product. 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 online at www.mn-net.com.

Please contact Technical Service regarding information about any changes to the current user manual compared with previous revisions.

2 Product description

2.1 The basic principle

The **NucleoSpin® DNA RapidLyse** kit is designed for fast and efficient isolation of genomic DNA from cells and organs like liver, kidney, heart, muscle, spleen, and lung. Processing of mouse tail and ear clippings is also possible. Fresh, frozen, and ethanol-preserved samples can be used.

The **NucleoSpin® DNA RapidLyse** kit lyses samples in maximal one hour agitated incubation at 56 °C. This is enabled by a thoroughly designed lysing setup with well balanced parameters that comprise a special lysis buffer in combination with Liquid Proteinase K. An incubation over night or for several hours is not necessary.

2.2 Kit specifications

Kit specifications at a glance

Parameter	NucleoSpin® DNA RapidLyse
Technology	Silica membrane technology
Format	Mini spin column
Sample material	Fresh, frozen, dried, and ethanol preserved tissue samples (e.g., organs), eukaryotic cells
Sample amount	Up to 40 mg fresh weight (sample dependent)
Typical yield	1–30 µg (depending on sample source)
A_{260}/A_{280}	1.7–1.9
Elution volume	60–100 µL
Preparation time	25 min (6 preps, excluding lysis)
Lysis time	Maximal 1 h
Binding capacity	60 µg

2.3 Handling, preparation, and storage of starting materials

Fresh, frozen, and ethanol preserved samples can be used. Make sure not to use more than 40 mg sample.

2.4 Lysis of sample material

In order to obtain optimal DNA yields and a smooth processing, sample material should be thoroughly lysed.

Lysis time depends upon sample material and may vary from a couple of minutes to one hour.

Sample material	Lysis time (optimal)	DNA yield (typical)	Specification
Cells	15 min	5 µg	10 ⁶ Hela cells
Bacteria (Gram-negative)	15 min	9–10 µg	30 mg <i>Pseudomonas fluorescens</i> (wet weight)
Bacteria (Gram-positive)	60 min	5 µg	30–40 mg <i>Corynebacterium glutamicum</i> (wet weight)
Blood	30 min	1 µg	200 µl EDTA whole blood
Organs (kidney)	60 min	30 µg	10 mg mouse kidney

Table 1 Optimal lysis time and typical yield for different samples types.

Genomic DNA was isolated with the NucleoSpin® DNA RapidLyse kit from the following: 10⁶ Hela cells; 30 mg Gram-negative bacteria *Pseudomonas fluorescens*; 30–40 mg Gram-positive bacteria *Corynebacterium glutamicum*, and 200 µl whole blood treated with EDTA. DNA was measured via OD after extraction according to the protocol for fresh, frozen and ethanol-preserved samples. *Note: For 200 µl blood samples 2 x binding buffer RLB was used.*

Most samples can be processed according to procedure 5.1. However, some sample materials (e.g., spleen or lung) need to be processed according to procedure 5.2 which requires additional material (see section 5.2 and 6.2).

2.5 Elution procedures

In addition to the standard method, several modifications are possible to increase yield, concentration, and convenience.

- **Convenient elution (standard elution):** Elution can be performed by a single addition of 100 µL Elution Buffer onto the column.
- **High yield:** Elution can be performed in two serial elutions of 100 µL each, resulting in a total volume of 200 µL.
- **High concentration:** Elution can be performed by application of 100 µL Elution Buffer, which is then re-used in a second elution step, resulting in 100 µL eluate with a high DNA concentration. Alternatively, the elution volume can be reduced down to 60 µL.

3 Storage conditions and preparation of working solutions

Attention:

Binding Buffer RLB contains chaotropic salts! Wear gloves and goggles!

CAUTION: Buffer RLB contains chaotropic salt which can form highly reactive compounds when combined with bleach (sodium hypochlorite). DO NOT add bleach or acidic solutions directly to the sample-preparation waste.

- All kit components can be stored at room temperature (18–25 °C) and are stable for at least one year.

Prior to the **NucleoSpin® DNA RapidLyse** procedure, prepare the following:

- **Wash Buffer RLW:** Add the indicated volume of ethanol (96–100 %) to **Wash Buffer RLW Concentrate**. Mark the label of the bottle to indicate that ethanol was added. Wash Buffer RLW can be stored at room temperature (18–25 °C) for at least one year.
- **Liquid Proteinase K** is ready to use. After first time use, store **Liquid Proteinase K** at 4 °C or -20 °C.

NucleoSpin® DNA RapidLyse			
REF	10 preps 740100.10	50 preps 740100.50	250 preps 740100.250
Wash Buffer RLW (Concentrate)	6 mL Add 24 mL ethanol	12 mL Add 48 mL ethanol	3 x 25 mL Add 100 mL ethanol to each bottle

4 Safety instructions

The following components of the **NucleoSpin® DNA RapidLyse** kits contain hazardous contents.

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

GHS classification

Only harmful features do not need to be labeled with H and P phrases up to 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
RLB	Guanidinium thiocyanate 30–45 % and ethanol 5–20 % <i>Guanidinthiocyanat 30–45 % und Ethanol 5–20 %</i> CAS 593-84-0, 64-17-5	 WARNING ACHTUNG	H226, H302, H412	P210, P264W, P273, P301+312, P330
Proteinase K	Proteinase K, liquid 1–3 % <i>Proteinase K flüssig 1–3 %</i> CAS 39450-01-6	 WARNING ACHTUNG	317	261sh, 280sh

Hazard phrases

H226	Flammable liquid and vapour. <i>Flüssigkeit und Dampf entzündbar.</i>
H302	Harmful if swallowed. <i>Gesundheitsschädlich bei Verschlucken.</i>
H317	May cause an allergic skin reaction. <i>Kann allergische Hautreaktionen verursachen.</i>
H412	Harmful to aquatic life with long lasting effects. <i>Schädlich für Wasserorganismen, mit langfristiger Wirkung.</i>

Precaution phrases

P210	Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking. <i>Von Hitze, heißen Oberflächen, Funken, offenen Flammen sowie anderen Zündquellenarten fernhalten. Nicht rauchen.</i>
P261sh	Avoid breathing dust/vapors. <i>Einatmen von Staub/Dampf vermeiden.</i>
P264W	Wash with water thoroughly after handling. <i>Nach Gebrauch mit Wasser gründlich waschen.</i>
P273	Avoid release to the environment. <i>Freisetzung in die Umwelt vermeiden.</i>

P280sh	Wear protective gloves/eye protection. <i>Schutzhandschuhe/Augenschutz tragen.</i>
P301+312	IF SWALLOWED: Call a POISON CENTER/ doctor/.../ if you feel unwell. <i>BEI VERSCHLUCKEN: Bei Unwohlsein GIFTINFORMATIONSZENTRUM/Arzt/... anrufen.</i>
P302+352	IF ON SKIN: Wash with plenty of water/... <i>BEI BERÜHRUNG MIT DER HAUT: Mit viel Wasser/... waschen.</i>
P330	Rinse mouth. <i>Mund ausspülen.</i>

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



The symbol shown on labels refers to further safety information in this section.
Das auf Etiketten dargestellte Symbol weist auf weitere Sicherheitsinformationen dieses Kapitels hin.

5 Protocols

5.1 Protocol for fresh, frozen, and ethanol-preserved samples

Before starting the preparations:

- Check if Buffer RLW was prepared according to section 3.
-

1 Lyse sample

Place the sample into a 2 mL tube.

Note: Do not use 1.5 mL conical tubes. The shape of the tube will impair thorough mixing. Use common 2 mL tubes which will facilitate proper sample and lysis buffer agitation.

Add 150 µL Buffer RLY.

Note: While mechanical homogenization of the sample is unnecessary in most cases, for some materials (e.g. fibrous tissue) a homogenization step in Buffer RLY prior to lysis may be beneficial for obtaining an optimal yield.

Add 10 µL Liquid Proteinase K.

Incubate at 56 °C on a heated shaking device (e.g. thermomixer) at maximum speed for a maximum time of 1 hour, or until the sample appears visually lysed (e.g. mostly cleared of particulates).

Note: If the sample is incubated in a heated water bath or heating block without agitation, vortex the sample frequently to ensure optimal lysis conditions.

Make sure that the tissue sample is submerged in the lysis buffer during incubation!

Centrifuge the tube at 11,000 x g for approx. 5 s (short spin), in order to clean the lid.

Note: If unlysed sample material remains after lysis, an additional centrifugation step is recommended to recover a cleared lysate. In this case, centrifuge 30 s at 14,000 x g.



+ 150 µL RLY
+ 10 µL Liquid
Proteinase K

2 Adjust DNA binding conditions

Add 440 µL Buffer RLB and mix (e.g., vortex 3 s).



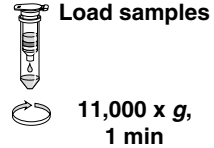
+ 440 µL RLB
Mix

3 Bind DNA

Apply the mixture (ca. 640 µL) onto the **NucleoSpin® DNA RapidLyse Column** placed into a 2 mL Collection Tube (provided).

Centrifuge for **1 min** at **11,000 x g**.

Discard Collection Tube with flow through. Put column into a fresh 2 mL Collection Tube (provided).



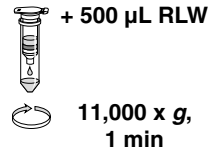
4 Wash silica membrane

1st wash

Add **500 µL Buffer RLW**.

Centrifuge for **1 min** at **11,000 x g**.

Discard flowthrough and place column back into the Collection Tube.

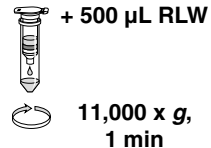


2nd wash

Add **500 µL Buffer RLW**.

Centrifuge for **1 min** at **11,000 x g**.

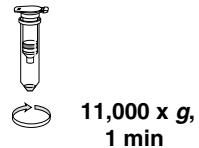
Discard flowthrough and place column back into the Collection Tube.



5 Dry silica membrane

Centrifuge for **1 min** at **11,000 x g**.

Note: Residual wash buffer is removed in this step.



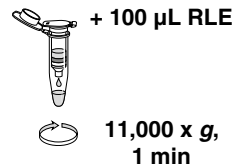
6 Elute highly pure DNA

Place the NucleoSpin® DNA RapidLyse Column into a 1.5 mL nuclease-free tube (not provided) and add **100 µL Buffer RLE** onto the column.

Centrifuge for **1 min** at **11,000 x g**.

Note: DNA yield can be increased by an incubation for 4 min at room temperature before centrifugation.

For alternative elution procedures see section 2.5.



5.2 Protocol for challenging samples (e.g., spleen and lung)

Before starting the preparations:

- The following items are additionally required for this protocol: MN Bead Tube Holder, Bead Tubes Type F (see ordering information).
- Check if Buffer RLW was prepared according to section 3.

1 Lyse sample

Place the sample into a **Bead Tube Type F**.

Add **120 µL Buffer RLE**.

Add **20 µL Buffer RLB**.

Add **10 µL Proteinase K**.

Insert the Bead Tube into the **MN Bead Tube Holder** and **shake 20 min** at **full speed** on a Vortex-Genie® 2. Up to 30 mg of wet weight sample can be processed.

Note: The use of other disruption devices is not recommended in conjunction with Bead Tube Type F. Due to the lysing matrix (corundum and steel beads) high impact disruption devices will cause steel abrasion and possible demolition of the bead tubes!



+ 120 µL RLE
+ 20 µL RLB
+ 10 µL Liquid Proteinase K

**Shake 20 min,
full speed**

2 Adjust DNA binding conditions

Add **420 µL Buffer RLB** and **mix** (e.g., vortex 3 s).

Centrifuge the tube at **11,000 x g** for approx. **5 s** (short spin), in order to clean the lid and sediment the lysing matrix.

DO NOT centrifuge for longer times and/or higher g-force, as this might damage the Bead Tubes due to the high density of the steel beads.



+ 420 µL RLB
Mix



**11,000 x g,
5 s**

3 Bind DNA

Apply cleared supernatant (approximately 500 µL) onto the **NucleoSpin® DNA RapidLyse Column** placed into a 2 mL Collection Tube (provided).

Note: Do not disturb the lysing matrix. Make sure not to transfer corundum matter from the lysing tube onto the column!

Centrifuge for **1 min** at **11,000 x g**.

Discard Collection Tube with flow through. Put column into a fresh 2 mL Collection Tube (provided).



Load samples



**11,000 x g,
1 min**

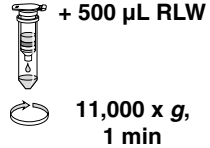
4 Wash silica membrane

1st wash

Add **500 µL Buffer RLW**.

Centrifuge for **1 min** at **11,000 x g**.

Discard flowthrough and place column back into the Collection Tube.

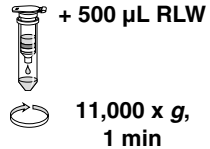


2nd wash

Add **500 µL Buffer RLW**.

Centrifuge for **1 min** at **11,000 x g**.

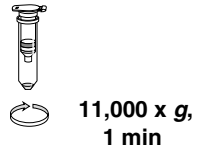
Discard flowthrough and place column back into the Collection Tube.



5 Dry silica membrane

Centrifuge for **1 min** at **11,000 x g**.

Note: Residual wash buffer is removed in this step.

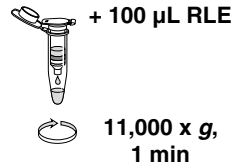


6 Elute highly pure DNA

Place the NucleoSpin® DNA RapidLyse Column into a 1.5 mL nuclease-free tube (not provided) and add **100 µL Buffer RLE** onto the column.

Centrifuge for **1 min** at **11,000 x g**.

For alternative elution procedures see section 2.5.



6 Appendix

6.1 Troubleshooting

Problem	Possible cause and suggestions
Grayish lysate or membrane	<i>Lysis with Bead Tube Type F for 20 min on the MN Bead Tube Holder might cause a slight grayish color of the lysate, which is tolerable. Prolonged shaking or use of other disruption devices can cause steel abrasion.</i> • Do not perform prolonged incubation, do not use other disruption devices with Bead Tube Type F.
Clogged column	<i>Too much sample material used</i> • Reduce the sample amount or follow procedure 5.2 for the next preparation. • Increase centrifugation time.
No or poor DNA yield	<i>Reagents not applied properly</i> • Prepare Buffer RLW according to the instructions (section 3). <i>Suboptimal elution of DNA from the column</i> • For certain sample types, preheat Buffer RLE to 70 °C before elution. Apply Buffer RLE directly onto the center of the silica membrane. • Elution efficiencies decrease dramatically if elution is done with buffers at a pH < 7.0. Use slightly alkaline elution buffers like Buffer RLE (pH 8.5). • Especially when expecting high yields from large amounts of material, we recommend elution with 200 µL RLE and incubation of the closed columns in an incubator at 70 °C for 5 min before centrifugation.

Problem	Possible cause and suggestions
Poor DNA quality	<i>High A_{260}/A_{280} ratio</i>
	Ratios > 1.9 can be caused by RNA contamination. Usually, such RNA contamination does not interfere with downstream applications. Depending on sample type, amount, and disruption procedure, preparations might contain small amounts of RNA. If it is necessary to reduce RNA contamination to the lowest possible level, incubate the lysate after disruption for 5 min at 70 °C in order to inactivate the Proteinase K. After cooling to room temperature, add 20 µL RNase A (20 mg/mL) and incubate 5 min. Continue with the application of the lysate onto the column.
	<i>Reagents not applied properly</i>
	Prepare Buffer RLW according to instructions (see section 3).
Suboptimal performance of gDNA in enzymatic reactions	<i>Carry-over of impurities</i>
	Residual liquid can be removed from the lid at any step of the protocol by an additional brief centrifugation step (approx. 1 s at 2,000 x g).
	<i>Carry-over of ethanol or salt</i>
	- Make sure to centrifuge ≥ 1 min at 11,000 x g in order to remove all of ethanolic Buffer RLW before eluting the DNA. - If, for any reason, the level of Buffer RLW has reached the column outlet after drying, repeat the centrifugation.
	<i>Contamination of DNA with inhibitory substances</i>
	Do not elute DNA with TE buffer. EDTA may inhibit enzymatic reactions. Re-purify DNA and elute in Buffer BE.

6.2 Ordering information

Product	REF	Pack of
NucleoSpin® DNA RapidLyse	740100.10/.50/.250	10/50/250 preps
NucleoSpin® DNA Insect	740470.10/.50	10/50 preps
NucleoSpin® Soil	740780.10/.50/.250	10/50/250 preps
NucleoSpin® DNA Stool	740472.10/.50/.250	10/50/250 preps
NucleoSpin® DNA Lipid Tissue	740471.10/.50	10/50 preps

Product	REF	Pack of
NucleoSpin® Microbial DNA	740235.10 / .50	10 / 50 preps
MN Bead Tube Holder	740469	1 piece
NucleoSpin® Bead Tubes Type A (0.6–0.8 mm ceramic beads, recommended for soil and sediments)	740786.50	50 pieces
NucleoSpin® Bead Tubes Type B (40–400 µm glass beads, recommended for bacteria)	740812.50	50 pieces
NucleoSpin® Bead Tubes Type C (1–3 mm corundum, recommended for yeast)	740813.50	50 pieces
NucleoSpin® Bead Tubes Type D (3 mm steel beads, recommended for insects)	740814.50	50 pieces
NucleoSpin® Bead Tubes Type E (40–400 µm glass beads and 3 mm steel beads, recommended for hard-to- lyse bacteria within insect samples)	740815.50	50 pieces
NucleoSpin® Bead Tubes Type F (1–3 mm corundum and 3 mm steel beads, recommended for challenging samples in conjunction with NucleoSpin® DNA RapidLyse – use only with MN Bead Tube Holder)	740816.50	50 pieces
NucleoSpin® Bead Tubes Type G (5 mm steel beads, recommended for plant material)	740817.50	50 pieces
Liquid Proteinase K	740396	5 mL
RNase A	740505 740505.50	50 mg 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® DNA RapidLyse 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

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Last updated: 07/2010, Rev. 03

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A054648/0190.5