This product distributed by
Takara Bio USA, Inc.

Ordering information
1.800.662.2566
ordersUS@takarabio.com

Technical support
1.800.662.2566
techUS@takarabio.com

TaKaRa
Clontech TaKaRa cellartis
takarabio.com

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Circulating DNA from plasma

User manual

NucleoSpin® 96 cfDNA
NucleoSpin® 96 cfDNA Core Kit

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MACHEREY-NAGEL
www.mn-net.com

Contact MN

Germany and international
MACHEREY-NAGEL GmbH & Co. KG
Neumann-Neander-Str. 6–8 · 52355 Düren · Germany
Tel.: +49 24 21 969-0
Toll-free: 0800 26 16 000 (Germany only)
Fax: +49 24 21 969-199
E-mail: info@mn-net.com

Technical Support Bioanalysis
Tel.: +49 24 21 969-270
E-mail: tech-bio@mn-net.com

USA
MACHEREY-NAGEL Inc.
2850 Emrick Blvd. · Bethlehem, PA 18020 · USA
Tel.: +1 484 821 0984
Toll-free: 888 321 6224 (MACH)
Fax: +1 484 821 1272
E-mail: sales-us@mn-net.com

France
MACHEREY-NAGEL SARL à associé unique
1, rue Gutenberg · 67722 Hoerdt · France
Tel.: +33 388 68 22 68
Fax: +33 388 51 76 88
E-mail: sales-fr@mn-net.com

Switzerland
MACHEREY-NAGEL AG
Hirsackerstr. 7 · 4702 Oensingen · Switzerland
Tel.: +41 62 388 55 00
Fax: +41 62 388 55 05
E-mail: sales-ch@mn-net.com

www.mn-net.com

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1 Components

1.1 Kit contents

	NucleoSpin® 96 cfDNA	
REF	**1 x 96 preps 740873.1**	**4 x 96 preps 740873.4**
Activation Buffer PMA	75 mL	2 x 100 mL
Lysis Buffer PML	100 mL	4 x 100 mL
Binding Buffer PMB	2 x 250 mL	8 x 250 mL
Wash Buffer PMW1	100 mL	500 mL
Wash Buffer PMW2 (Concentrate) *	50 mL	2 x 100 mL
Elution Buffer PME **	13 mL	60 mL
Liquid Proteinase K	6 mL	4 x 6 mL
NucleoSpin® cfDNA Binding Plate (red rings)	1	4
MN Wash Plate	1	4
Square-well Block (96 wells)	2	8
Round-well Block with Cap Strips	1	4
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* For preparation of working solutions and storage conditions see section 3

** Elution Buffer PME: 5 mM Tris/HCl, pH 8.5

	NucleoSpin® 96 cfDNA Core Kit	
REF	**1 x 96 preps 740874.1**	**4 x 96 preps 740874.4**
Activation Buffer PMA	75 mL	2 x 100 mL
Lysis Buffer PML	100 mL	4 x 100 mL
Binding Buffer PMB	2 x 250 mL	8 x 250 mL
Wash Buffer PMW1	100 mL	500 mL
Wash Buffer PMW2 (Concentrate) *	50 mL	2 x 100 mL
Elution Buffer PME	13 mL	60 mL
Liquid Proteinase K	6 mL	4 x 6mL
NucleoSpin® cfDNA Binding Plate	1	4
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1.2 Reagents, consumables, and equipment to be supplied by user

Reagents:

- 96–100 % ethanol

Consumables:

- For lysis and adjusting of binding conditions: 1 x 96 Square-well Block for processing 500 µL samples, 2 x 96 Square-well Blocks for processing 1 mL samples, 4 x 96 Square-well Blocks for processing 2 mL samples. Alternatively, 24-Square-well Blocks can be used (see ordering information section 6.2).
- Disposable pipette tips

Equipment:

- NucleoVac 96 Vacuum Manifold (see ordering information section 6.2)
- NucleoVac Vacuum Regulator (see ordering information section 6.2)
- Vacuum pump
- Heater-shaker or incubator oven
- Multi channel pipettes or large volume pipettes with appropriate tips
- Personal protection equipment (lab coat, gloves, goggles)

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

** Elution Buffer PME: 5 mM Tris/HCl, pH 8.5

1.3 About this user manual

It is strongly recommended for first time users to read the detailed protocol sections of the **NucleoSpin® 96 cfDNA** 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***.

2 Product description

2.1 The basic principle

The **NucleoSpin® 96 cfDNA** kit is designed for the efficient isolation of circulating DNA from human blood plasma. Fragmented DNA of 50 bp and larger can be purified with high efficiency. The **NucleoSpin® 96 cfDNA** kit can be used with manual and automated vacuum manifolds. The kit is fully automatable on many liquid handling robots.

The protocol follows state-of-the-art bind-wash-elute procedures: lysis is performed within 30 minutes with Proteinase K and lysis buffer. Afterwards, a binding buffer is added and the solution is applied onto the wells of the binding plate in several steps and DNA is bound to the silica membrane. Three washing steps efficiently remove contaminating substances, such as PCR inhibitors. Drying of silica is achieved by applying vacuum and pure DNA is finally eluted.

2.2 Kit specifications

- The **NucleoSpin® 96 cfDNA** kit is recommended for the isolation of circulating cell-free DNA from human plasma.
- The **NucleoSpin® 96 cfDNA** kit is designed for high recovery of fragmented DNA ≥ 50 bp.
- Up to 2 mL plasma can be used as sample material in a single well
- DNA yield strongly depends on the individual sample, but is typically in the range of 0.1 to 100 ng DNA per mL plasma.
- DNA is ready to use for downstream applications such as real-time PCR or NGS.
- The preparation time is approximately 90 min for up to 96 plasma samples.

Kit specifications at a glance

Parameter	NucleoSpin® 96 cfDNA
Technology	Silica-membrane technology
Format	96-well plates
Sample material	Human EDTA / Cell-Free DNA BCT® (Streck) plasma
Sample amount	0.5–2 mL per preparation
Typical yield	sample dependent
Elution volume	100 µL (recovered volume about 70 µL)
Preparation time	approx. 90 min / 96 preps

2.3 Size and yield of DNA from plasma

Usually, DNA concentrations in plasma are in a range of 0.1 ng up to several 100 ng DNA per mL of plasma. The amount of circulating DNA in plasma depends on health condition of the donor, sampling and handling of the blood, plasma preparation, DNA isolation method, and others.

A significant portion of the cell-free DNA in plasma originates from apoptotic cells. Therefore, a considerable percentage of this circulating DNA is known to be highly fragmented. However, the degree of fragmentation and the ratio of fragmented DNA to high molecular weight DNA depends on several parameters like origin of the DNA (e.g., fetal, tumor, microbial DNA), health condition of the blood donor, blood sampling procedure, and handling of the sample.

2.4 Handling of sample material

Circulating DNA yield and quality is largely influenced by blood sampling technique, handling, storage, and plasma preparation. It is highly recommended to perform these steps as uniform as possible in order to achieve highest reproducibility.

Plasma can be isolated according to the following recommendation:

Preparation of plasma from human EDTA blood or Streck Cell-Free DNA BCT®

1 Centrifuge samples for 10 min at 2,000 x *g*.

2 Remove the plasma without disturbing sedimented cells and particles.

3 Clear plasma of residual cellular debris by means of centrifugation (10 min at 5000 x *g*).

4 If necessary, freeze plasma samples in fresh tubes. Upon thawing, check for precipitates and remove them with a final centrifugation step.

2.5 Elution procedures

The recommended standard elution procedure comprises two steps of 50 µL. This will result in about 70 µL eluate. The retained volume will contain very little amounts of DNA because the majority will be present in the eluted fraction.

2.6 Stability of isolated DNA

Due to the low DNA content in plasma and the resulting low total amount of isolated DNA, its fragmentation, and the absence of DNase inhibitors (the elution buffer does NOT contain EDTA), the eluates should be kept on ice for short term storage and frozen at -20 °C for long term storage.

3 Storage conditions and preparation of working solutions

Attention: *Buffers PML, PMB and PMW1 contain guanidinium hydrochloride (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. Wear gloves and goggles!*

Storage conditions:

- All kit components can be stored at room temperature (18–25 °C) and are stable for at least one year.
- If there is any precipitate present in the buffers, warm the buffer up to 25–37 °C to dissolve the precipitate before use.

Before starting any **NucleoSpin® 96 cfDNA** protocol, prepare the following:

- **Wash Buffer PMW2**: Ethanol has to be added to Wash Buffer PMW2 (Concentrate) according to the instructions on the label and in this user manual. Mark the label of the bottle to indicate that ethanol was added. All other kit components are ready to use.
- Prepare plasma sample according to section 2.4.
- Set heating block or incubator oven to 56 °C for lysis.
- Set up the **NucleoVac 96 Vacuum Manifold**.
- **Liquid Proteinase K** is ready to use. After first opening, store Liquid Proteinase K at 4 °C or -20 °C
- When using multi well plates, samples have to be split into suitable aliquots.

	NucleoSpin® 96 cfDNA	
REF	**1x 96 preps** **740873.1**	**4 x 96 preps** **740873.4**
Buffer PMW2 (Concentrate)	50 mL Add 200 mL ethanol	2 x 100 mL Add 400 mL ethanol to each bottle

	NucleoSpin® 96 cfDNA Core Kit	
REF	**1 x 96 preps** **740874.1**	**4 x 96 preps** **740874.4**
Buffer PMW2 (Concentrate)	50 mL Add 200 mL ethanol	2 x 100 mL Add 400 mL ethanol to each bottle

4 Safety instructions

The following components of the **NucleoSpin® 96 cfDNA** and **NucleoSpin® 96 cfDNA Core Kit** 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 *Inhalt*	Hazard contents *Gefahrstoff*	GHS symbol *GHS-Symbol*	Hazard phrases *H-Sätze*	Precaution phrases *P-Sätze*
Activation Buffer PMA	Sodium hydroxide solution 0.5–1.0 % *Natriumhydroxid-Lösung 0,5–1,0 %* CAS 1310-73-2	 WARNING *ACHTUNG*	315, 319	280sh
Lysis Buffer PML	Guanidine hydrochloride 50–66 % *Guanidinhydrochlorid 50–66 %* CAS 50-01-1	 WARNING *ACHTUNG*	302, 315, 319	264W, 280sh, 301+312, 330
Binding Buffer PMB	Guanidine hydrochloride 24–36 % and ethanol 35–55 % *Guanidinhydrochlorid 24–36 % und Ethanol 35–55 %* CAS 50-01-1, 64-17-5	 WARNING *ACHTUNG*	226, 302	210, 264W, 301+312, 330
Wash Buffer PMW1	Guanidine hydrochloride 36–50 % and 2-propanol 20–35 % *Guanidinhydrochlorid 36–50 % und 2-Propanol 20–35 %* CAS 50-01-1, 67-63-0	 WARNING *ACHTUNG*	226, 302, 319, 336	210, 260D, 264W, 280sh, 301+312, 330

Hazard phrases

H 226	Flammable liquid and vapor *Flüssigkeit und Dampf entzündbar.*
H 302	Harmful if swallowed. *Gesundheitsschädlich bei Verschlucken.*
H 315	Causes skin irritation. *Verursacht Hautreizungen.*
H 317	May cause an allergic skin reaction. *Kann allergische Hautreaktionen verursachen.*

H 319	Causes serious eye irritation. *Verursacht schwere Augenreizung*
H 336	May cause drowsiness or dizziness. *Kann Schläfrigkeit und Benommenheit verursachen.*

Precaution phrases

P 210	Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking. *Von Hitze, heissen Oberflächen, Funken, offenen Flammen sowie anderen Zündquellenarten fernhalten. Nicht rauchen*
P 260D	Do not breathe vapors. *Dampf nicht einatmen.*
P 264W	Wash with water thoroughly after handling. *Nach Gebrauch mit Wasser gründlich waschen.*
P 280sh	Wear protective gloves/eye protection. *Schutzhandschuhe/Augenschutz tragen.*
P 301+312	IF SWALLOWED: Call a POISON CENTER/doctor if you feel unwell. *BEI VERSCHLUCKEN: Bei Unwohlsein GIFTINFORMATIONSZENTRUM/Arzt anrufen.*
P330	Rinse mouth. *Mund ausspülen.*

 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 Protocol for the isolation of DNA from plasma

Protocol at a glance

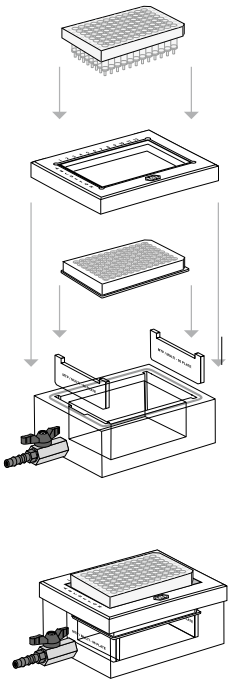
1 Lyse samples	**25 µL Proteinase K** **1 mL plasma** **Mix** **400 µL Buffer PML** **Mix** **Incubate at 56 °C, 30 min** *Note: For Streck Cell-Free DNA BCT® incubate 60 min*
	Prepare the NucleoVac 96 Vacuum Manifold
	400 µL Buffer PMA per well **-0.4 bar*, 1 min**
2 Adjust DNA binding conditions	**2 mL Buffer PMB** **Mix**
3 Bind DNA	**Transfer lysates** *Note: Transfer lysates in aliquots of 1 mL* **-0.4 bar*, 2 min**
4 Wash silica membrane	**800 µL PMW1** **-0.4 bar*, 2 min** **1 mL PMW2** **-0.4 bar*, 2 min** **1 mL PMW2** **-0.4 bar*, 2 min**
5 Dry silica membrane	**-0.6 bar*, 10 min**

* Reduction of atmospheric pressure

6 Elute DNA	**50 µL PME** **Incubate 1 min at RT** **-0.4 bar*, 30 s** **50 µL PME** **-0.6 bar*, 30 s**

Setup of vacuum manifold:

Binding / Washing steps



Step 4:
Place the NucleoSpin® cfDNA Binding Plate on top of the manifold lid.

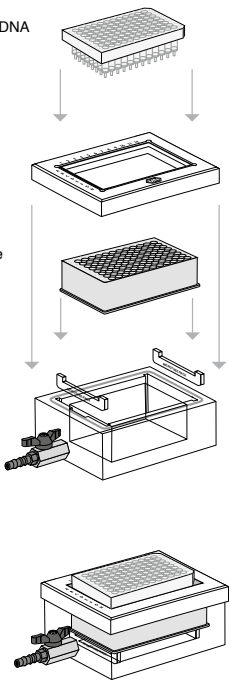
Step 3:
Place the manifold lid on top of the manifold base.

Step 2:
Place the MN Wash Plate in the manifold.

Step 1:
Insert spacers 'MTP/MULTI-96 PLATE' and waste container in the manifold base.

Final setup

Elution step



Step 4:
Place the NucleoSpin® cfDNA Binding Plate on top of the manifold lid.

Step 3:
Place the manifold lid on top of the manifold base.

Step 2:
Place the Round-well Block in the manifold.

Step 1:
Insert spacers 'ROUNDWELL BLOCK' in the manifold base.

Final setup

* Reduction of atmospheric pressure

5.1 Detailed protocol

The procedure below describes the isolation of cell-free DNA from 1 mL human plasma. Adjusting reagent volumes according to the table below allows for processing of plasma volumes from 0.5–2 mL.

Plasma volume [mL]	Liquid Proteinase K [µL]	Lysis Buffer PML [µL]	Binding Buffer PMB [mL]
0.5	12.5	200	1
1	25	400	2
2	50	800	4

1 Lyse sample

Add **25 µL Liquid Proteinase K** to each well of a 24-Square-well block. (Alternatively, samples can be split into aliquots of 500 µL and processed in 96 Square-well Blocks.)

Add **1 mL plasma** and **mix** by pipetting.

Add **400 µL Buffer PML** and **mix** by pipetting.

Incubate at **56 °C** for **30 min** (For Streck Cell-Free DNA BCT®, incubate 60 min; ideally with shaking).

Prepare the NucleoVac 96 Vacuum manifold

Insert spacers 'MTP/MULTI-96 PLATE', the Waste Container and the Wash Plate into the manifold base. Place the manifold lid on top and then the **NucleoSpin® cfDNA Binding Plate**.

While incubating the lysis, apply **400 µL Buffer PMA** to the wells of the NucleoSpin® cfDNA Binding Plate. Incubate one minute, then apply vacuum of **-0.4 bar*** for **1 min**.

2 Adjust binding conditions

Add **2 mL Buffer PMB** to each well and mix by pipetting.

3 Bind DNA

Transfer prepared lysates to the NucleoSpin® cfDNA Binding Plate in aliquots of 1 mL.

Apply vacuum of **-0.4 bar*** for **2 min**.

Lysates can be loaded continuously while they are passing the membrane. Remove, empty and replace Waste Container after the third loading step as well as after column loading is completed.

* Reduction of atmospheric pressure

4 Wash silica membrane

1st wash

Once all lysates have passed the membrane, add **800 µL Buffer PMW1** to each well. Incubate for **1 min**, and then apply vacuum of **-0.4 bar*** for **2 min**.

2nd wash

Add 1 mL **Buffer PMW2**, and then apply vacuum of **-0.4 bar*** for **2 min**.

3rd wash

Repeat 2nd wash step.

Remove and empty Waste Container. Remove Wash Plate.

5 Dry silica membrane

Apply strongest possible vacuum of at least **-0.6 bar*** for **10 min** to dry the silica membrane.

After drying, blot column outlets on tissue paper to remove residual ethanol.

Insert spacers "ROUNDWELL Block" and place a Round-well Block on top. Place the manifold lid on top and then the **NucleoSpin® cfDNA Binding Plate.**

6 Elute highly pure DNA

Add **50 µL Buffer PME** (first elution step) to the membrane. Incubate **1 min**.

Apply vacuum of **-0.4 bar*** for **30 s**.

Add **50 µL Buffer PME** (second elution step) to the membrane.

Apply vacuum of **-0.6 bar*** for **30 s**.

Spin eluates down and cover elution plate.

* Reduction of atmospheric pressure

6 Appendix

6.1 Troubleshooting

Problem	Possible cause and suggestions
Low DNA yield	*Low DNA content of the sample* • The content of cell-free DNA in human plasma may vary over several orders of magnitude. DNA contents in the range of 0.1–1000 ng DNA per mL of plasma have been reported (see remarks in section 2.3). • If the DNA concentration is measured with double strand specific dyes, e.g., PicoGreen®, make sure not to heat the DNA before measurement. Due to denaturation of DNA during the heat incubation step and the double strand specificity of certain DNA dyes, e.g., PicoGreen®, results may be inaccurate.
Column clogging	*Sample contains residual cell debris or cells* • The plasma sample may have contained residual cells or cell debris. Make sure to use only clear plasma samples (see remarks in section 2.4).
Discrepancy between A_{260} quantification values and PCR quantification values	*Silica abrasion from the membrane* • Due to the typically low DNA content in plasma and the resulting low total amount of isolated DNA, DNA quantification via A_{260} absorption measurement is often hampered due to the low sensitivity of the absorption measurement. When performing absorption measurements close to the detection limit of the photometer, the measurement may be influenced by minor amounts of silica abrasion. In order to prevent incorrect A_{260}-quantification of small DNA amounts, centrifuge the eluate for 30 s at > 11.000 x *g* and take an aliquot for measurement without disturbing any sediment. Alternatively, use a silica abrasion insensitive DNA quantification method (e.g., PicoGreen® fluorescent dye).
Unexpected A_{260}/A_{280} ratio	*Measurement not in the range of photometer detection limit* • In order to obtain a significant A_{260}/A_{280} ratio, it is necessary that the initially measured A_{260} and A_{280} values are significantly above the detection limit of the photometer used. An A_{280} value close to the background noise of the photometer will cause unexpected A_{260}/A_{280} ratios.

6.2 Ordering information

Product	REF	Pack of
NucleoSpin® 96 cfDNA	740873.1 740873.4	1 4
NucleoSpin® 96 cfDNA Core Kit	740874.1 740874.4	1 4
24-Square-well Block U-Bottom	740448.4 740448.24	4 24
Square-well Block (96 wells)	740481 740481.24	4 24
Round-well Block with Cap-strips	740475 740475.24	4 24
Lysis Buffer PML	740835.125	125 mL
Binding Buffer PMB	740836.250	250 mL
Liquid Proteinase K	740396	5 mL
NucleoVac 96 Vacuum Manifold	740681	1
NucleoVac Vacuum Regulator	740641	1

Note: The product has been formerly distributed under the name NucleoSpin® 96 DNA Plasma. The product code (REF) and kit content have not been changed.

6.3 Product use restriction / warranty

NucleoSpin® 96 cfDNA 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.

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

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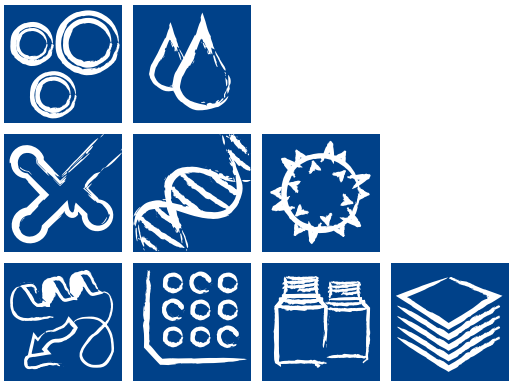


Plasmid DNA

Clean up

RNA

DNA

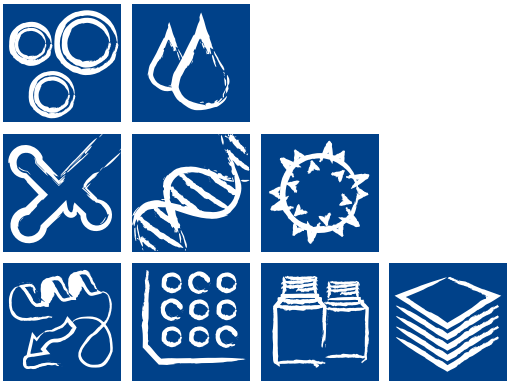
Viral RNA and DNA

Protein

High throughput

Accessories

Auxiliary tools



www.mn-net.com

MACHEREY-NAGEL

MACHEREY-NAGEL GmbH & Co. KG · Neumann-Neander-Str. 6–8 · 52355 Düren · Germany

DE / International:
Tel.: +49 24 21 969-0
Fax: +49 24 21 969-199
E-mail: info@mn-net.com

CH:
Tel.: +41 62 388 55 00
Fax: +41 62 388 55 05
E-mail: sales-ch@mn-net.com

FR:
Tel.: +33 388 68 22 68
Fax: +33 388 51 76 88
E-mail: sales-fr@mn-net.com

US:
Tel.: +1 484 821 0984
Fax: +1 484 821 1272
E-mail: sales-us@mn-net.com

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