Clean Pathogen DNA & RNA Kit

Instructions for Use

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For Research Use Only

CPT-DR0096, CPT-DR0384

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Intended Use

Clean Pathogen is intended for use by professional users trained in molecular biology techniques. It is designed to use manually or on a liquid handling workstation for molecular biology applications.

Introduction and Principle

The Clean Pathogen DNA/RNA Kit is designed for high throughput and reliable isolation of both DNA and RNA from tissue, serum and fecal samples.

The Clean Pathogen Kit will isolate DNA from:

- High quality host genomic DNA
- Gram positive and negative bacterial DNA
- Fungal spore DNA
- Viral DNA

The Clean Pathogen Kit will isolate RNA from:

- Host RNA
- Viral RNA

The Clean Pathogen DNA/RNA Kit combines our propriety buffer system with the convenience of our magnetic CleanNA Particles CPT Our buffer system minimizes the binding of PCR inhibiting compounds, present within the samples, onto our magnetic particles.

The protocol is fully scalable and due to the use of our magnetic bead purification technology, can besides manual usage, easily be automated once the samples have been lysed on liquid handling workstations (e.g. Dynamic Devices LYNX™, Hamilton STAR™).

Purified DNA is suitable for PCR, qPCR, restriction digestion, Next Generation Sequencing and hybridization applications. Purified RNA is suitable for reverse transcription for cDNA synthesis, RT-PCR, RT-qPCR and RNA-Seq.

Schematic Overview

With our specially formulated lysis buffer in combination with the glass bead mix in the included disruptor plate, the samples are lysed. The DNA and RNA is bound to our magnetic particles while DNases and RNases are deactivated. DNA and RNA is isolated from the lysates in one step by binding to the CleanNA Particles' surfaces. The CleanNA magnetic particles are separated from the lysates by using a magnetic separation device. Following a few rapid wash steps to remove trace contaminants, the purified DNA/RNA is eluted from the CleanNA particles for downstream applications using an Elution Buffer.

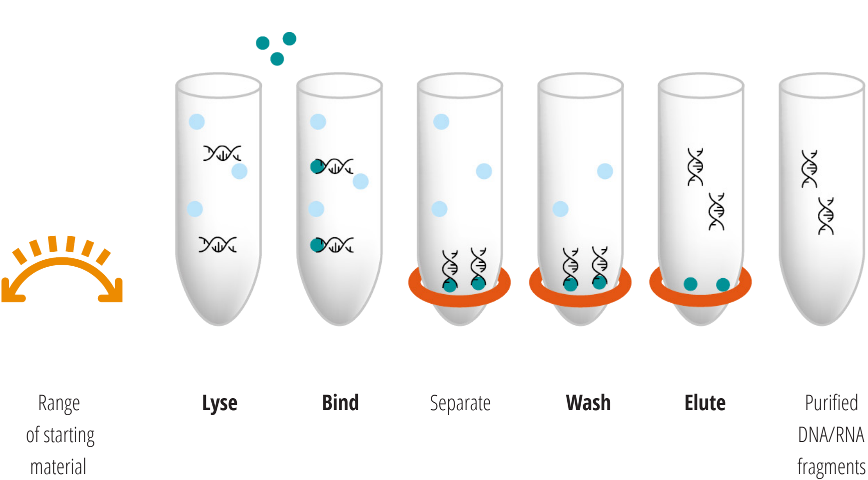

Figure 1: Schematic overview of the Clean Pathogen DNA & RNA Kit extraction procedure.

Materials Provided

Kit Contents:

Component	CPT-DR0096 (1x96 preps)	CPT-DR0384 (4x96 preps)
Clean Disruptor Plate	1	4
Caps	13	52
Lysis Buffer CPT	60 mL	240 mL
PK Buffer CPT	8 mL	30 mL
Proteinase K Solution	2.2 mL	9 mL
Binding Buffer CPT	40 mL	160 mL
CPT Prep Buffer	40 mL	160 mL
CleanNA Particles CPT	2.2 mL	9 mL
CPT Wash Buffer 1	88 mL	3 x 88 mL
CPT Wash Buffer 2	30 mL	4 x 30 mL
Elution Buffer	15 mL	50 mL

Reagent Shipping, Storage and Handling

Clean Pathogen DNA & RNA Kit is shipped at room temperature (15-25 °C). Do not freeze the components of the Clean Pathogen DNA & RNA Kit. After the components have been frozen, the kit is no longer suitable for use. Do not use the Clean Pathogen DNA & RNA Kit after the expiration date stated on the outer box label.

Component	Storage Temperature
Clean Disruptor Plate	15-25 °C
Caps	15-25 °C
Lysis Buffer CPT	15-25 °C
PK Buffer CPT	15-25 °C
Proteinase K Solution	15-25 °C (for storage > 12 months, store at 2-8 °C)
Binding Buffer CPT	15-25 °C
CPT Prep Buffer	15-25 °C
CleanNA Particles CPT	2-8 °C
CPT Wash Buffer 1	15-25 °C
CPT Wash Buffer 2	15-25 °C
Elution Buffer	15-25 °C

⚠ **Note:** Check all buffers for precipitates prior to usage. Any precipitates can be re-dissolved by warming the buffer(s) to 37ºC and shaking gently.

Warnings

Read the instructions carefully before using the kit.

Do not mix several kit LOT numbers.

The LOT number on the CleanNA Particles CPT box packaging is different from the LOT number on the CleanNA Particles CPT bottle. The LOT number on the box matches the LOT number of the whole kit and the one on the bottle is specifically for the particles. Since the CleanNA Particles CPT are stored at a different temperature than the rest of the kit, please make sure that the LOT number on the box packaging of the particles matches the LOT number of the kit before use.

Precautions

When working with chemicals, always follow your facility's procedures and universal precautions by using disposable gloves, safety glasses, a labcoat etc.

For all safety information, please consult the safety data sheet (SDS).

BT Lysis Buffer	
	Harmful if swallowed. Causes skin irritation. Causes serieus eye irritation. Toxic if inhaled. Avoid breathing dust/fume/gas/mist/vapours/spray. **IF IN EYES:** Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. continu rinsing. Call a POISOIN CENTER or doctor/ physician.
BT Binding Buffer	
	May cause fire or explosion; strong oxidizer. May be harmful if swallowed. Wear protective gloves/ protective clothing/ eye protection/ face protection. **IF IN EYES:** Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER or doctor/ physician.
Proteinase K Solution	
	May cause an allergic skin reaction. Causes skin irritation. Causes serious eye irritation. May cause allergy or asthma symptoms or breathing difficulties if inhaled. May cause respiratory irritation. Wear protective gloves/protective clothing/eye protection/face protection. Avoid breathing dust/fume/gas/mist/vapours/spray. **IF IN EYES:** Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. If experiencing respiratory symptoms: Call a POISON CENTER or doctor/physician.
BT Wash Buffer 1	
	Harmful if swallowed. Causes skin irritation. Causes serious eye irritation. Toxic if inhaled. Avoid breathing dust/fume/gas/mist/vapours/spray. **IF IN EYES:** Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Call a POISON CENTER or doctor/physician.

Note: For safe disposal, please consult your local waste regulations.

Quality Control

CleanNA produces each lot of Clean Pathogen DNA & RNA Kit according to predetermined and validated protocols in the Quality Management System (QMS). Additionally, a quality check after production of each lot is performed to secure consistent product quality. CleanNA's QMS is EN-ISO 13485 certified.

Materials and Equipment to be Supplied by User

Materials and reagents to be supplied by user for Tissue and Serum & Stool protocol:

- Centrifuge capable of at least 3,500 x g with adaptor for 96-well plates
- Magnetic separation device for 96-well plates (CleanNA, Part# CMAG-96-RN50)
- Incubator capable of 70°C
- 96-well plates with a capacity of at least 1.7 mL (Recommend Nunc 278752) and compatible with the Magnetic Separation Device
- 96-well microplates for DNA storage
- Vortexer
- Absolute ethanol
- Molecular biology grade water
- Optional: Mixer mill such as a SPEX CertiPrep Geno/Grinder® 2010 or Qiagen TissueLyser

Materials and reagents to be supplied by user for Urine & Whole Blood protocol:

- Centrifuge capable of at least 3,500 x g with adaptor for 96-well plates
- Magnetic separation device for 96-well plates (CleanNA, Part# CMAG-96-RN50)
- Incubator capable of 70°C
- 96-well plates with a capacity of at least 1.7 mL (Recommend Nunc 278752) and compatible with the Magnetic Separation Device
- 96-well microplates for DNA storage
- For processing Whole Blood: 2 mL screw cap tubes
- Vortexer
- Absolute ethanol
- Molecular biology grade water
- Optional: Mixer mill such as a SPEX CertiPrep Geno/Grinder® 2010 or Qiagen TissueLyser
- Ice Bucket

Working RNase Free

For RNA applications it is important to work RNase free. RNases are present everywhere and general precautions should be taken to avoid the introduction of RNases and other contaminating nucleases while working with RNA. The most common sources of RNases are hands, dust particles and contaminated laboratory solutions, equipment and glassware.
To minimize the risk of RNase contamination we recommend the following precautions:

- Always use gloves when handling RNA samples. Change your gloves frequently, to avoid contaminations;
- Ensure to use RNase free filter tips for pipetting;
- Use materials such as disposable consumables, which are guaranteed RNase free;
- Use reagents which are guaranteed RNase free. Creating aliquots from buffers lowers the risk of RNase contamination in buffers, reagents, etc.;
- Avoid using reagents, consumables and equipment dedicated for common use or general lab processes;
- If possible work in a separate room, fume hood or lab space;
- Clean all working surfaces with commercial RNase inhibiting surfactant or 70% ethanol before starting your work / experiment.

Preparation of Reagents

Wash Buffer 1

Prepare CPT Wash Buffer 1 with absolute ethanol as follows and store at room temperature.

Kit	Absolute ethanol to be added
CPT-DR0096	112 mL
CPT-DR0384	112 mL per bottle

Wash Buffer 2

Dilute CPT Wash Buffer with absolute ethanol as follows and store at room temperature.

Kit	Absolute ethanol to be added
CPT-DR0096	70 mL
CPT-DR0384	70 mL per bottle

Shake or vortex the CleanNA Particles CBT to fully resuspend the particles prior to usage. The particles must be fully suspended during use to ensure proper binding.

Tissue Protocol

This protocol describes the procedure for the isolation of both host and pathogen DNA & RNA from tissue samples.

Before Starting:

• Prepare CPT Wash Buffer 1 and CPT Wash Buffer 2 according to the “Preparation of Reagents” section on Page 11.
• Set an incubator to 70°C.
• Heat Elution Buffer to 70°C.

Protocol:

1. Briefly spin the Clean Disruptor Plate to remove any glass beads from the walls of the wells. Uncap the Clean Disruptor Plate and save the caps for use in Step 3.

2. Add 25-30 mg tissue to each well.

3. Add 525 μL Lysis Buffer CPT to each sample. Seal the Clean Disruptor Plate with the caps removed in Step 1.

4. Vortex at maximum speed for 3-5 minutes to lyse and homogenize samples. For best results, a Mixer Mill, such as Spex CertiPrep Geno/Grinder® 2010 or Qiagen Tissuelyser, should be used.

⚠ **Note:** Depending on the sample amount and type, the amount of Lysis Buffer CPT may need to be adjusted so that 300 μL can be recovered after Step 11.

5. Centrifuge at 1,000-2,000 x g for 60 seconds at room temperature.

6. Remove and discard the caps from the Clean Disruptor Plate.

7. Add 53 μL PK Buffer CPT and 20 μL Proteinase K Solution to each sample.

8. Seal the Clean Disruptor Plate with new Caps for Racked Microtubes (provided).

9. Vortex for 60 seconds to mix thoroughly.

10. Incubate at 70°C for 15 minutes. Mix once during incubation.

11. Centrifuge at 3,500 x g for 10 minutes.

12. Transfer 300 μL cleared supernatant to a 96-well deep-well plate (1.7 mL) compatible with the Magnetic Separation Device used.

⚠ **Note:** Do not transfer any debris as it can reduce yield and purity.

13. Add 300 µL Binding Buffer CPT, 300 µL CPT Prep Buffer, and 20 µL CleanNA Particles CPT to each sample. Vortex to mix thoroughly or pipet up and down 20 times.

⚠ **Note:** CleanNA Particles CPT and Binding Buffer CPT can be prepared as a master mix prior to use. Prepare only what is needed. Tip mixing is recommended for automated protocols for best yield.

14. Incubate at room temperature for 10 minutes.
15. Place the 96-well deep-well plate on the Magnetic Separation Device to magnetize the CleanNA Particles CPT. Incubate at room temperature until the CleanNA Particles CPT are completely cleared from solution.
16. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles CPT.
17. Remove the plate containing the CleanNA Particles CPT from the Magnetic Separation Device.
18. Add 600 µL CPT Wash Buffer 1 to each sample. Resuspend the CleanNA Particles CPT by vortexing or pipetting up and down 20 times.

⚠ **Note:** CPT Wash Buffer 1 must be diluted with absolute ethanol prior to use. Please see Page 11 for instructions.

19. Incubate at room temperature for 2 minutes.
20. Place the 96-well deep-well plate on the Magnetic Separation Device to magnetize the CleanNA Particles CPT. Incubate at room temperature until the CleanNA Particles CPT are completely cleared from solution.
21. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles CPT.
22. Remove the plate containing the CleanNA Particles CPT from the Magnetic Separation Device.
23. Repeat Steps 18-22 once for a second CPT Wash Buffer 1 step.
24. Add 600 µL CPT Wash Buffer 2 to each sample. Resuspend the CleanNA Particles CPT by vortexing or pipetting up and down 20 times.

⚠ **Note:** CPT Wash Buffer 1 must be diluted with absolute ethanol prior to use. Please see Page 11 for instructions.

25. Incubate at room temperature for 2 minutes.
26. Place the 96-well plate on the Magnetic Separation Device to magnetize the CleanNA Particles CPT. Incubate at room temperature until the CleanNA Particles

CPT are completely cleared from solution.

27. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles CPT.

28. Leave the plate on the Magnetic Separation Device.

29. Add 500 µL molecular biology grade water (not provided) to each sample. Immediately aspirate and remove the molecular biology grade water. Do not let the samples stay in contact with the molecular biology grade water for more than 60 seconds.

⚠ **Note:** This step (water "wash") is crucial to remove traces of ethanol from the sample. When adding water, the CleanNA Particles CC will come out of the ring shape shortly. This is a normal phenomenon, the CleanNA particles CC will return to the ring shape after a few seconds.

Note: If using an automated platform, use the maximum volume the tips will allow up to 600 µL.

30. Add 50-100 µL Elution Buffer heated to 70°C to each sample. Resuspend CleanNA Particles CPT by vortexing or pipetting up and down 20 times.

31. Incubate at room temperature for 5 minutes.

32. Place the 96-well plate on the Magnetic Separation Device to magnetize the CleanNA Particles CPT. Incubate at room temperature until the CleanNA Particles CPT are completely cleared from solution.

33. Transfer the cleared supernatant containing purified DNA/RNA to a clean 96-well microplate. Store the DNA at -20°C. For downstream RNA applications, store the eluate at -80°C.

Serum & Stool Protocol

This protocol describes the procedure for the isolation of both host and pathogen DNA & RNA from serum and stool samples.

Before Starting:

• Prepare CPT Wash Buffer 1 and CPT Wash Buffer 2 according to the “Preparation of Reagents” section on Page 11.
• Set an incubator to 70°C.
• Heat Elution Buffer to 70°C.

Protocol:

1. Briefly spin the Clean Disruptor Plate to remove any glass beads from the walls of the wells. Uncap the Clean Disruptor Plate and save the caps for use in Step 3.
2. Add 250 µL serum or stool samples to each well. If stool sample is solid, resuspend to 10% wgt/volume in PBS before starting.
3. Add 275 µL Lysis Buffer CPT to each sample. Seal the Clean Disruptor Plate with the caps removed in Step 1.
4. Vortex at maximum speed for 3-5 minutes to lyse and homogenize samples. For best results, a Mixer Mill, such as Spex CertiPrep Geno/Grinder® 2010 or Qiagen Tissuelyser, should be used.

⚠ **Note:** Depending on the sample amount and type, the amount of Lysis Buffer CPT may need to be adjusted so that 300 µL can be recovered after Step 11.

5. Centrifuge at 1,000-2,000 x g for 60 seconds at room temperature.
6. Remove and discard the caps from the Clean Disruptor Plate.
7. Add 50µL PK Buffer CPT and 20 µL Proteinase K Solution to each sample.
8. Seal the Clean Disruptor Plate with new Caps for Racked Microtubes (provided).
9. Vortex for 60 seconds to mix thoroughly.
10. Incubate at 70°C for 15 minutes. Mix once during incubation.
11. Centrifuge at 3,500 x g for 10 minutes.
12. Transfer 300 µL cleared supernatant to a 96-well deep-well plate (1.7 mL)

compatible with the Magnetic Separation Device used.

⚠ **Note:** Do not transfer any debris as it can reduce yield and purity.

13. Add 300 µL Binding Buffer CPT, 300 µL CPT Prep Buffer, and 20 µL CleanNA Particles CPT to each sample. Vortex to mix thoroughly or pipet up and down 20 times.

⚠ **Note:** CleanNA Particles CPT and Binding Buffer CPT can be prepared as a master mix prior to use. Prepare only what is needed. Tip mixing is recommended for automated protocols for best yield.

14. Incubate at room temperature for 10 minutes.

15. Place the 96-well deep-well plate on the Magnetic Separation Device to magnetize the CleanNA Particles CPT. Incubate at room temperature until the CleanNA Particles CPT are completely cleared from solution.

16. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles CPT.

17. Remove the plate containing the CleanNA Particles CPT from the Magnetic Separation Device.

18. Add 600 µL CPT Wash Buffer 1 to each sample. Resuspend the CleanNA Particles CPT by vortexing or pipetting up and down 20 times.

⚠ **Note:** CPT Wash Buffer 1 must be diluted with absolute ethanol prior to use. Please see Page 11 for instructions.

19. Incubate at room temperature for 2 minutes.

20. Place the 96-well deep-well plate on the Magnetic Separation Device to magnetize the CleanNA Particles CPT. Incubate at room temperature until the CleanNA Particles CPT are completely cleared from solution.

21. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles CPT.

22. Remove the plate containing the CleanNA Particles CPT from the Magnetic Separation Device.

23. Repeat Steps 18-22 once for a second CPT Wash Buffer 1 step.

24. Add 600 µL CPT Wash Buffer 2 to each sample. Resuspend the CleanNA Particles CPT by vortexing or pipetting up and down 20 times.

⚠ **Note:** CPT Wash Buffer 2 must be diluted with absolute ethanol prior to use. Please see Page 11 for instructions.

25. Incubate at room temperature for 2 minutes.

26. Place the 96-well plate on the Magnetic Separation Device to magnetize the CleanNA Particles CPT. Incubate at room temperature until the CleanNA Particles CPT are completely cleared from solution.

27. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles CPT.

28. Leave the plate on the Magnetic Separation Device.

29. Add 500 µL molecular biology grade water (not provided) to each sample. Immediately aspirate and remove the molecular biology grade water. Do not let the samples stay in contact with the molecular biology grade water for more than 60 seconds.

⚠ **Note:** This step (water "wash") is crucial to remove traces of ethanol from the sample. When adding water, the CleanNA Particles CC will come out of the ring shape shortly. This is a normal phenomenon, the CleanNA particles CC will return to the ring shape after a few seconds.

Note: If using an automated platform, use the maximum volume the tips will allow up to 600 µL.

30. Add 50-100 µL Elution Buffer heated to 70°C to each sample. Resuspend the CleanNA Particles CPT by vortexing or pipetting up and down 20 times.

31. Incubate at room temperature for 5 minutes.

32. Place the 96-well plate on the Magnetic Separation Device to magnetize the CleanNA Particles CPT. Incubate at room temperature until the CleanNA Particles CPT are completely cleared from solution.

33. Transfer the cleared supernatant containing purified DNA/RNA to a clean 96-well microplate. Store the DNA at -20°C. For downstream RNA applications, store the eluate at -80°C.

Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact your local distributor.

Possible problems and Suggestions

Problem	Cause	Suggestion
Low DNA yield or no DNA yield	Poor homogenization of sample.	Repeat the DNA isolation with a new sample, be sure to mix the sample with Lysis Buffer CPT thoroughly. Use a commercial homogenizer if possible.
	Incomplete resuspension of CleanNA Particles CPT.	Resuspend the CleanNA Particles CPT by vortexing vigorously before use.
	DNA washed off.	Make sure CPT Wash Buffer 1 and CPT Wash Buffer 2 are mixed with ethanol.
		Ensure the water "wash" does not exceed 60 seconds and the CleanNA Particles CPT are not resuspended.
	CleanNA Particles CPT lost in process.	After water is added during wash step CleanNA Particles CPT will go into solution. Let magnetic particles re-magnetize prior to aspirating liquid.
cDNA synthesis not working	Degraded RNA.	Store the eluate at -80°C after isolation and minimize the number of freeze thaw cycles.
		Ensure to work RNase free and use RNase free materials during the isolation process and in downstream applications.
CleanNA Particles CPT do not completely clear from solution	Magnetizing time too short.	Increase the particle collection time on the magnetic separation device.
A260/A230 ratio is low	Salt contamination.	Repeat the DNA isolation with a new sample. Extend the incubation time with CPT Wash Buffer 1. Wash the CleanNA Particles CPT with ethanol.
A260/A280 ratio is high	RNA contamination.	The protocol does not remove RNA. If desired, add 5 µL RNase A (25 mg/mL) after lysate is cleared and before binding buffers are added. Incubate at room temperature for 5 minutes.

Problem	Cause	Suggestion
Problems in downstream applications	BSA not added to PCR mixture.	Add BSA to a final concentration of 0.1 µg/mL to the PCR mixture.
	Too much DNA inhibits PCR reactions.	Dilute the DNA elute used in the downstream application if possible.
	Non-specific bands in downstream PCR.	Use hot-start Taq polymerase mixture.
	Inhibitory substance in the eluted DNA.	Check the A260/A230 ratio. Dilute the elute to 1:50 if necessary.
Abnormal BioAnalyzer data	BioAnalyzer shows multiple sharp peaks during the analysis.	Store the eluate at -80°C after isolation and minimize the number of freeze thaw cycles.
		Ensure to work RNase free and use RNase free materials during the isolation process and in downstream applications.
	BioAnalyzer shows base line climbing towards the end.	Check the BioAnalyzer chip for air bubbles. Load samples onto a new freshly prepared chip.
	BioAnalyzer shows high blob at the beginning of the trace.	Ensure the purified sample does not contain traces of CleanNA Particles CC.

Symbols

REF	Reference number
	Manufacturer
	Caution
	Temperature limit
	Expiration date
LOT	Lot number

Ordering Information

Contact your local distributor to order.

Product	Part Number
Clean Pathogen DNA & RNA Kit 96 preps	CPT-DR0096
Clean Pathogen DNA & RNA Kit 384 preps	CPT-DR0384

Product	Part Number
Clean Magnet Plate 96-well RN50	CMAG-96-RN50

Document Revision History

Manual Version	Date of revision	Revised Chapter	Explanation of revision
8	29/FEB/2024	Materials Provided and Urine and Whole Blood protocol	Removed Urine and Whole Blood protocol from manual.
7	27/OCT/2023	Total revision.	New lay-out. Added chapters schematic overview, warnings, pre-cautions, quality control, symbols.
6.00	October 2021	Total revision.	Language and layout modifications.
		Kit contents and material.	Added CPT+ Reagent to table.
5.00	August 2020	Total revision.	New lay-out.
			Important general information added at page 1 (before contents).

Notes

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