

**Catalog Numbers:**

CPT-DR0096: 96 preps

CPT-DR0384: 384 preps

**Batch No:** See package**Shipping:** Room temperature**Storage and stability:** CleanNA Particles CPT should be stored at 4°C upon receipt, store all other components at room temperature. See page 3 for more storage information.**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.

## USER MANUAL

Manual revision v6.00

**Quality Control:** Each lot of Clean Pathogen is tested against predetermined specifications to ensure consistent product quality. If in any case inconsistencies occur, please contact us at [info@cleanna.com](mailto:info@cleanna.com) or +31 (0) 182 22 33 50.**Safety precautions:** When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. Please refer to the material safety data sheet for further information.**Emergency:** In case of a medical emergency due to the use of this product, contact your local poison control center. When a severe incident occurs, please inform CleanNA at +31 (0) 182 22 33 50 or [info@cleanna.com](mailto:info@cleanna.com).**Expiry:** When stored under the recommended conditions and handled correctly, full activity is retained until the expiry date on the outer box label.**FOR RESEARCH USE ONLY**

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## Contents

Introduction and Principle .....	2
Kit Contents and Materials .....	3
Working RNase Free.....	4
Preparation of Reagents .....	4
Tissue Protocol.....	5
Serum & Stool Protocol.....	7
Urine and Whole Blood Protocol .....	9
Troubleshooting Guide .....	12
Ordering Information.....	13
Document Revision History.....	14
Notes.....	15

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## Introduction and Principle

The Clean Pathogen DNA/RNA Kit is designed for high throughput and reliable isolation of both DNA and RNA from tissue, urine, whole blood, 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.

With our specially formulated lysis buffer, samples are lysed and 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.

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.

## Kit Contents and Materials

### Kit Contents:

Product	CPT-D0096	CPT-D0384	Storage
Preps	1 x 96	4 x 96	n/a
Clean Disruptor Plate	1	4	15-25°C
Caps	13	52	15-25°C
Lysis Buffer CPT	60 mL	240 mL	15-25°C
PK Buffer CPT	8 mL	30 mL	15-25°C
Proteinase K Solution	2.2 mL	9 mL	15-25°C (for storage > 12 months, store at 2-8°C)
Binding Buffer CPT	40 mL	160 mL	15-25°C
CPT Prep Buffer	40 mL	160 mL	15-25°C
CPT+ Reagent	25 mL	100 mL	15-25°C
CleanNA Particles CPT	2.2 mL	9 mL	2-8°C
CPT Wash Buffer 1	88 mL	3 x 88 mL	15-25°C
CPT Wash Buffer 2	30 mL	4 x 30 mL	15-25°C
Elution Buffer	15 mL	50 mL	15-25°C

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

### 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-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
- 100% 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-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
- 100% 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 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
CPT-DR0096	112 mL
CPT-DR0384	112 mL per bottle

### Wash Buffer 2

Dilute CPT Wash Buffer 2 with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
CPT-DR0096	70 mL
CPT-DR0384	70 mL per bottle

Shake or vortex the CleanNA Particles CPT 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 4.
- 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  $\mu$ L 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  $\mu$ L 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  $\mu$ L PK Buffer CPT and 20  $\mu$ L 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  $\mu$ L 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  $\mu$ L Binding Buffer CPT, 300  $\mu$ L CPT Prep Buffer, and 20  $\mu$ L 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  $\mu$ L 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 100% ethanol prior to use. Please see Page 4 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  $\mu$ L 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 100% ethanol prior to use. Please see Page 4 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  $\mu$ L 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  $\mu$ L.

30. Add 50-100  $\mu$ L 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 4.
- 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  $\mu\text{L}$  serum or stool samples to each well. If stool sample is solid, resuspend to 10% wgt/volume in PBS before starting.
3. Add 275  $\mu\text{L}$  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  $\mu\text{L}$  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 50 $\mu\text{L}$  PK Buffer CPT and 20  $\mu\text{L}$  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  $\mu\text{L}$  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  $\mu\text{L}$  Binding Buffer CPT, 300  $\mu\text{L}$  CPT Prep Buffer, and 20  $\mu\text{L}$  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  $\mu\text{L}$  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 100% ethanol prior to use. Please see Page 4 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  $\mu\text{L}$  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 100% ethanol prior to use. Please see Page 4 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  $\mu\text{L}$  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  $\mu\text{L}$ .

30. Add 50-100  $\mu\text{L}$  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.



# Urine and Whole Blood Protocol

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

## Before Starting:

- Prepare CPT Wash Buffer 1 and CPT Wash Buffer 2 according to the “Preparation of Reagents” section on Page 4.
- Set an incubator to 70°C
- Heat Elution Buffer to 70°C
- Prepare an ice Bucket

## 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. Sample addition:
  - a. For urine, add 250 µL urine samples to each well of the Clean Disruptor plate
  - b. For Whole Blood, transfer 250 µL sample into a clean screw cap tube (not provided). To each screw cap tube, add the content of 1 Clean Disruptor Plate tube, ensuring all glass beads are added to the tube.



**Note:** Whole Blood needs to be processed in a screw cap tube in order to prevent cross contamination, since it will foam quite intensively during homogenization.

3. Add 275 µL Lysis Buffer CPT to each sample. Seal the Clean Disruptor Plate (for urine) with the caps removed in Step 1. For Whole Blood close the screw cap tube, using its screw cap.
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 12.

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 or the screw cap tubes.
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) or re-use the screw cap from the screw cap tube.
9. Vortex for 60 seconds to mix thoroughly.
10. Incubate at 70°C for 15 minutes. Mix once during incubation.
11. Add 200 µL CPT+ Reagent to each well. Place the plate on ice for 5 minutes.
12. Centrifuge at 3,500 x g for 10 minutes.
13. Transfer 300 µL cleared supernatant to a 96-well deep-well plate (1.2 mL) compatible with the Magnetic Separation Device used.



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

14. Add 300  $\mu\text{L}$  Binding Buffer CPT, 300  $\mu\text{L}$  CPT Prep Buffer, and 20  $\mu\text{L}$  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.

15. Incubate at room temperature for 10 minutes.
16. 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.
17. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles CPT.
18. Remove the plate containing the CleanNA Particles CPT from the Magnetic Separation Device.
19. Add 600  $\mu\text{L}$  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 100% ethanol prior to use. Please see Page 4 for instructions.

20. Incubate at room temperature for 2 minutes.
21. 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.
22. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles CPT.
23. Remove the plate containing the CleanNA Particles CPT from the Magnetic Separation Device.
24. Repeat Steps 19-23 once for a second CPT Wash step.
25. Add 600  $\mu\text{L}$  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 100% ethanol prior to use. Please see Page 4 for instructions.

26. Incubate at room temperature for 2 minutes.
27. 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.
28. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles CPT.
29. Leave the plate on the Magnetic Separation Device.
30. Add 500  $\mu\text{L}$  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  $\mu\text{L}$ .

31. Add 50-100  $\mu\text{L}$  Elution Buffer heated to  $70^{\circ}\text{C}$  to each sample. Resuspend the CleanNA Particles CPT by vortexing or pipetting up and down 20 times.
32. Incubate at room temperature for 5 minutes.
33. 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.
34. Transfer the cleared supernatant containing purified DNA/RNA to a clean 96-well microplate. Store the DNA at  $-20^{\circ}\text{C}$ . For downstream RNA applications, store the eluate at  $-80^{\circ}\text{C}$ .

## Troubleshooting Guide

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

### Possible problems and Solutions

Problem	Cause	Solution
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	Too short of magnetizing time.	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.

## Troubleshooting Guide

Problem	Cause	Solution
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.	Ensure to remove all traces of the cleared supernatant after each wash step.
		Ensure to perform a water “wash” to remove final ethanol traces.
	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.

## 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-RN50

## Document Revision History

Manual Version	Date of revision	Revised Chapter	Explanation of revision
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 layout.
			Important general information added at page 1 (before contents).

## Notes



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