CARBON
TECHNOLOGIES

Viral DNA/RNA Isolation
Based on Silica Technology
MiniPrep

For Viral DNA/RNA Isolation from
Body Fluid
Serum
Plasma
Animal Tissue

Contents

Kit Content

Component	100 Preps, PR242001
GLB	25ml
TLB	18ml
BWB1 (concentrate)	2 x 16ml
BWB2 (concentrate)	2 x 12ml
ERR	20ml
RJ-Protease	2 x 1.25ml
Carrier RNA	620µg
HiPure DR Column	100
Collection Tube	200

Storage

Shipment condition is checked by Carbon Technologies. After arrival, all reagents should be kept dry, at room temperature. We suggest storing RJ-Protease at 2-8°C, and for routine use, it is recommended that you aliquot it to 100µl volumes and storage at 2-8°C. Also, Carrier RNA is storable at room temperature before preparation. However, after adding ERR buffer, it is recommended that it must store at -20°C and be aliquoted to 100µl volumes and avoid frequent freeze-thaw. When storage condition is as directed, all reagents are stable until expiration date, as indicated on the kit box.

Intended Use

DNall VirAll Kit provides the components and procedures necessary for purifying viral DNA/RNA from cell-free samples such as body fluid, serum, plasma and animal tissue. Notice that, DNall VirAll Kit is intended for molecular biology applications not for diagnostic use. We recommend all users to study RNA experiments guideline, before starting their work.

Guarantee & Warranty

Carbon Technologies guarantees the efficiency of all manufactured kits and reagents. For more information on choosing proper kits based on your needs, please contact our technical support team. If any product does not satisfy you, due to reasons other than misuse, please contact our technical support team. If problem is due to manufacturing process, Carbon Technologies will replace the kit for you.

Warning and Precautions

Due to chemical material usage that may be hazardous, always make sure to wear suitable lab coat, disposable gloves, and protective eyewear. Material Safety Data Sheet (MSDS) for all products and reagents are provided. They are accessible online at www.carbontechnologiesco.com.

Quality Control

DNall VirAll Kit is tested against predetermined experiments on a lot-to-lot basis according to Carbon Technologies ISO-certified quality management system, to ensure consistent product quality. For your information, the results of all experiments are accessible by addressing Cat and Lot number at www.carbontechnoloesco.com.

Description

The DNall VirAll Kit is designed for rapid and efficient purification of high-quality viral DNA/RNA from various human and animal liquid samples such as body fluid, plasma, serum and animal tissue. The kit utilizes a silica-based membrane technology in the form of a convenient spin column for nucleic acid isolation. DNall VirAll Kit needs less handling and it is convenient for simultaneous isolation, which makes it favorites for laboratory with many isolations in a day. The purified viral nucleic acids are free of proteins, nucleases, and other contaminants or inhibitors of downstream applications. Isolated DNA/RNA can be directly used in PCR, qPCR or other nucleic acid-based assays.

Procedure

DNall VirAll Kit is designed for isolating both viral DNA and RNA from body fluid, serum, plasma and genomic DNA from animal tissue. Lysis is achieved by incubation of the sample in GLB, for both DNA and RNA isolation; and in a RJ-Protease enzyme solution, just for viral DNA isolation. Appropriate conditions for DNA and RNA binding to the silica membrane is achieved by the addition of ethanol to the lysate. Then, DNA or RNA is selectively bound to the membrane. Contaminants are removed by two specific washing buffers. Pure viral DNA/RNA is finally eluted in rehydration buffer. Isolated DNA/RNA is ready to use in downstream applications. A symmetric peak at 260 nm by spectrophotometer, confirms high purity of isolated nucleic acid.

Equipment & Reagents required but not supplied

- Molecular biology grade ethanol (96-100%)
- Sterile, RNase-free pipets and pipet tips
- 1.5 ml Microtube
- Vortex
- Centrifuge and Micro centrifuge
- Dry Heat Block/ Water Bath

Applications

The isolated DNA/RNA can be used in many downstream applications:

- Different kinds of PCRs
- Viral genotyping
- Viral detection
- Viral load monitoring

Features

Specific features of DNall VirAll Kit are listed here in Table 1.

Table 1. DNall VirAll Kit features and specifications

Features	Specifications
Elution volume	20-200µl
Technology	Silica technology
Main sample type	Body Fluid, serum, Plasma, Animal tissue
Processing	Manual
Sample amount	-Plasma and serum: Up to 200µl -All tissue kind expect spleen: up to 25mg (Spleen up to 10mg)
Biomolecule isolation	DNA and RNA
Operation time per reaction	-Less than 30min (for viral DNA/RNA isolation) -Less than 20Min (for whole blood, buffy coat, serum and plasma) -Less than 2 h (for animal tissue and bacteria)
Typical yield	Varies
Carrier RNA	Provided in the kit
Enzyme	RJ-Protease

Sample Preparation

- If possible, use only fresh sample material. Do not freeze/thaw samples more than once.
- Plasma and serum samples can be stored at 2-8°C for up to 24 hours, or at −20°C or −70°C for long-term storage.
- Urine samples should be stored at 2-8°C for up to 12 hours (with 0.5 M EDTA added to 50 mM final concentration), or at −20°C or −70°C for long term storage (cells should be collected by centrifugation).
- Before use, equilibrate samples to room temperature (20±5°C). Remove precipitates from plasma/serum samples, if any, by centrifugation for 5min at 3,000 × g.
- Use EDTA or citrate treated plasma samples.
- Urogenital swabs can be stored at 2-8°C for up to 48 hours. For longer term storage cells should be collected by centrifugation and stored at −20°C or −70°C.
- Nasal and buccal swabs can be stored at 2-8°C for up to 48 hours.

Washing Buffer Preparation

Before the first use, add appropriate amount of ethanol (96-100%) to each washing buffer tube, then mix thoroughly

to prepare washing buffer, refer to Table 2. Do not forget to tick the check box on the bottle label to indicate that ethanol has been added. Before each use mix reconstituted buffer by shaking. Storing at room temperature.

Table 2: Washing buffer preparation

Buffer Name	Concentrated Volume	Amount of Ethanol	Final Volume
BWB1	16ml	24ml	40ml
BWB2	12ml	28ml	40ml

Protocol 1: Isolation of Viral DNA

Sample type:

- Body fluid
- Serum
- Plasma

Recommended Starting Material for Isolation of Viral DNA

The size of recommended starting material to use with determined lysis volume are listed here.

Table 3: Recommended starting material and Lysis Buffer amount

Sample	Size of Starting material	Lysis Buffer Amount
Plasma/serum	Up to 200µl	200µl

Some tips to know

- All centrifugation steps are carried out at room temperature (15–25°C).
- Do not forget to add the appropriate amount of molecular biology grade ethanol (96–100%) to BWB1 and WB2 buffers as indicated on the bottle, before using for the first time, refer to washing buffer preparation.
- If GLB forms precipitate, please warm it to 56°C until the precipitate has fully dissolved.

Process

- Add 25µl RJ-Protease to a 1.5ml clean microcentrifuge tube. Add 200µl sample (plasma, serum, body fluid and etc.) to the tube. Then add 200 µl GLB. Pulse vortex for 15 s and incubate at 56 °C for 12min.
- Add 200µl ethanol (96-100%) to the lysate, mix by pulse vortexing for 15 s, then centrifuge briefly.
- Gently, pipette the mixture to a HiPure DR column placed in a 2ml collection tube (supplied in the kit box). Centrifuge at 8000 rpm for 1min. Discard flow-through and place back the HiPure DR column in to the collection tube.
- Add 500µl BWB1 and centrifuge for 1min at 8000 rpm, discard the flow-through. Place back the HiPure DR column in to the collection tube.
- Add 500µl BWB2 and centrifuge for 3min at 14000 rpm. Discard both the flow-through and the collection tube. Place the HiPure DR column in a new clean 1.5ml microcentrifuge tube (not provided).

Note: To avoid ethanol carry over, be careful that the column does not come into contact with the flow-through, if it happens discard the flow-through, place the column back in a collection tube and centrifuge for another 1min at 14000 rpm.

- Pipette 30-50µl ERR directly onto HiPure DR column. Incubate at room temperature for 1-5min. Centrifuge it at 12000 rpm for 1min.

Protocol 2: Isolation of Viral Nucleic Acid (RNA/DNA)

Sample type:

- Body fluid
- Serum
- Plasma

Recommended Starting Material for Isolation of Viral Nucleic Acid (DNA/RNA)

The size of recommended starting material to use with determined lysis volume are listed here.

Table 4: Recommended starting material and Lysis Buffer amount

Sample	Size of Starting material	Lysis Buffer Amount
Plasma/serum	Up to 200µl	200µl

Some tips to know

- All centrifugation steps are carried out at room temperature (15–25°C).
- Equilibrate samples to room temperature.
- Equilibrate ERR to room temperature for elution step.
- Prepare a 56°C thermoblock for use.
- Add Carrier RNA reconstituted in ERR to GLB according to following instructions.
- For the first use, add molecular biology grade ethanol (96–100%) to the bottle containing BWB1 [1] concentrate, as described on the bottle. Store reconstituted BWB1 at room temperature (15–25°C), refer to washing buffer preparation.

  Note: Always mix reconstituted BWB1 by shaking before starting the procedure.
- For the first use, add molecular biology grade ethanol (96–100%) to the bottle containing BWB2 concentrate, as described on the bottle, refer to washing buffer preparation. Store reconstituted BWB2 at room temperature (15–25°C).

  Note: Always mix reconstituted BWB2 by shaking before starting the procedure.
- If GLB forms precipitate, please warm it to 56°C until the precipitate has fully dissolved.

Addition of carrier RNA to GLB2

Carrier preparation

[1] Contains chaotropic salt. Take appropriate laboratory safety measures and wear gloves when handling. Not compatible with disinfectants containing bleach

Add 620µl ERR to the tube containing 620 µg lyophilized carrier RNA to obtain a solution of 1µg/µl (for 5 Prep sample add 31µl ERR to the tube containing 31µg lyophilized carrier RNA to obtain a solution of 1 µg/µl). Dissolve the carrier RNA thoroughly, divide it into conveniently sized aliquots, and store it at –20°C. Do not freeze–thaw the aliquots of carrier RNA more than 3 times.

Important! Note that carrier RNA does not dissolve in GLB. It must first be dissolved in ERR and then added to GLB.

GLB preparation

Calculate the volume of Buffer GLB–carrier RNA mix needed per batch of samples to be simultaneously processed from Table 5.

Table 5: Volumes of GLB and carrier RNA–ERR mix required for the procedure

Samples Number	GLB amount (ml)	Carrier RNA–ERR amount(µl)	Samples Number	GLB amount (ml)	Carrier RNA–ERR amount (µl)
1	0.22	6.2	13	2.86	80.1
2	0.44	12.3	14	3.08	86.3
3	0.66	18.5	15	3.30	92.4
4	0.88	24.6	16	3.52	98.6
5	1.10	30.8	17	3.74	104.7
6	1.32	37.0	18	3.96	110.9
7	1.54	43.1	19	4.18	117.0
8	1.76	49.3	20	4.40	123.2
9	1.98	55.4	21	4.62	129.4
10	2.20	61.6	22	4.84	135.5
11	2.42	67.8	23	5.06	141.7
12	2.64	73.9	24	5.28	147.8

Attention! Mix gently by inverting the tube 10 times. To avoid foaming, do not vortex.

Note: The sample-preparation procedure is optimized for 5.6 µg of carrier RNA per sample. If less carrier RNA has been shown to be better for your amplification system, transfer only the required amount of dissolved carrier RNA to the tubes containing GLB.

Process

- Pipet 25µl RJ-Protease into a 1.5ml clean microcentrifuge tube. Add 200µl of sample (plasma, serum, body fluid etc.) into the tube.

  Note: If the sample volume is less than 200µl, add the appropriate volume of 0.9% sodium chloride solution to bring the total volume up to 225µl (including RJ-Protease).
- Add 200µl GLB (containing 28µg/ml of carrier RNA). Mix by pulse vortexing for 15s.

  Note: It is crucial that the sample and GLB are mixed thoroughly to yield a homogeneous solution.

  Note: Do not add RJ-Protease directly to GLB.

- Incubate at 56°C for 15min in a thermoblock. Then, centrifuge briefly to remove drops from the inside of the lid.
- Add 250µl of molecular biology grade ethanol (96–100%) to the sample; mix thoroughly by pulse vortexing for 15 s. Incubate the lysate with the ethanol for 5 min at room temperature (15–25°C).

  Note: Cool ethanol on ice before adding to the lysate, if ambient temperature exceeds 25 °C.
- Centrifuge the tube briefly to remove drops from the inside of the lid. Carefully pipette all of the lysate from previous step onto the HiPure DR column without wetting the rim.
- Centrifuge at 8000 rpm for 1min. Discard the flow-through and place back the HiPure DR column in the collection tube.

  Note: If the lysate did not pass the column, centrifuge at full speed until it passes through the column.
- Add 500µl BWB1 and centrifuge at 8000 rpm for 1min. Discard the flow-through and place back the HiPure DR column in the collection tube.

  Note: This step increases kit performance when processing inhibitory samples.
- Add 500µl BWB2 and centrifuge at 8000 rpm for 1min. Discard the flow-through and place back the HiPure DR column in the collection tube.
- Add 500µl molecular biology grade ethanol (96–100%) and centrifuge at 8000 rpm for 1min. Discard the flow-through.

  Attention! Ethanol carryover into the eluate may cause problems in downstream applications. Removing the HiPure DR column and collection tube from the rotor may also cause flow-through to come into contact with the HiPure DR column.
- Place back the HiPure DR column in the collection tube. Centrifuge at full speed 14000 rpm for 3min to dry the membrane completely. Then discard both the collection tube and flow-through.

  Recommended: Place the HiPure DR column into a new 2ml collection tube (not provided), open the lid, and incubate the assembly at 56 °C for 3min to dry the membrane completely.

  * This step serves to evaporate any remaining liquid.
- Place the HiPure DR column in a clean 1.5 ml clean microcentrifuge tube (not provided). Apply 20–150µl ERR to the center of the membrane. Close the lid and incubate at room temperature for 5min.
- Centrifuge at full speed, 14000 rpm for 1min.

  * Ensure that ERR is equilibrated to room temperature.

  Important! If elution is done in small volumes (<50µl), ERR must be dispensed onto the center of the membrane for complete elution of bound RNA and DNA.

  Note: Incubation after adding ERR for 5min at room temperature before centrifugation generally increases viral DNA and RNA yield.

Genomic DNA Isolation

Protocol 3: Isolation of Genomic DNA (Animal blood, Cells, Body fluid, serum, plasma)

Sample type:

- Animal blood
- Cells
- Body fluid
- Serum

- Plasma

Recommended starting material

To reach optimized results it is better to follow as listed here. The size of recommended material is written in Table 6.

Table 6: Appropriate size of starting material

Sample	Size of Starting Material
Blood*	Up to 250µl
Cultured cell	≤5 x 10^6 cells
Buffy Coat	Up to 200µl
Animal tissue	All tissues except spleen: up to 25 mg (spleen up to 10 mg)
Bacteria cells	2.5 x 10^8 to 2 x 10^9

*White Blood Cell counts are extremely variable from a person to person and therefore, for optimum results its recommended to determine cell counts for choosing the best protocol for purification. For healthy individuals, white blood cell counts normally from 4-10 million per 1ml blood; however, depending on disease state, cell counts may be higher or lower than the normal range.

Some tips to know

- All centrifugation steps are carried out at room temperature (15–25°C).
- Do not forget to add the appropriate amount of ethanol (96–100%) to BWB1 and BWB2 as indicated on the bottle, before using for the first time, refer to washing buffer preparation part.
- If GLB forms precipitate, please warm it to 56°C until the precipitate has fully dissolved.

Process

- Add 25µl RJ-Protease to a 1.5ml clean microcentrifuge tube. Add 250µl blood (plasma, serum, body fluid and etc.) to the tube. Then add 250µl GLB. Pulse vortex for 15 s and incubate at 56 °C for 12min.

  Note: For cell pellets, add 250 µl GLB directly to the pellet or alternatively, for direct lysis of cells grown in a monolayer, add the appropriate amount of GLB to the cell-culture dish. Collect the lysate with a rubber policeman. Transfer the lysate into a microcentrifuge tube. Vortex to mix, and ensure that no cell clumps are visible.

  Optional: This protocol is specialized for DNA isolation. For usual and routine applications RNase treatment isn't needed. If RNA-free genomic DNA is required, add 10µl Prime-RNase A, mix by vortexing, and incubate for 5min at room temperature before going to step 2.
- Add 250µl ethanol (96-100%) to the lysate, mix by pulse vortexing for 15 s, then centrifuge briefly.
- Gently, pipette the mixture to a HiPure DR column placed in a 2ml collection tube (supplied in the kit box). Centrifuge at 8000 rpm for 1min. Discard flow-through and place the HiPure DR column in new collection tube (provided in the kit box).

  Note: If the lysate did not pass the column, centrifuge at full speed until it passes through the column.

- Add 600µl BWB1 and centrifuge for 1min at 8000 rpm, discard the flow-through and place the HiPure DR column in the previous collection tube.
  - Add 600µl BWB2 and centrifuge for 3min at 14000 rpm. Discard both the flow-through and the collection tube. Place the HiPure DR column in a new clean 1.5ml microcentrifuge tube (not provided).

    Note: To avoid ethanol carry over, be careful that the column does not come into contact with the flow-through, if it happens discard the flow-through, place the column back in a collection tube and centrifuge for another 1min at 14000 rpm.
- Pipette 50-200µl ERR directly onto HiPure DR column. Incubate at room temperature for 1-5 min. Centrifuge it at 8000 rpm for 1min.

  Note: If the expected DNA yield is more than the yield from pervious step, put the HiPure DR column on a new microtube and add another 50-200µl ERR, incubate for 5min at room temperature. Then, centrifuge for 1min at 8000 rpm. However, it is possible to pass the follow-through from step 6 once more to obtain DNA with higher concentrations.

Protocol 4: Isolation of Genomic DNA (Buffy coat)

Sample type: Buffy coat

Some tips to know

- All centrifugation steps are carried out at room temperature (15–25°C).
- Do not forget to add the appropriate amount of ethanol (96–100%) to BWB1 and BWB2 as indicated on the bottle, before using for the first time, refer to washing buffer preparation part.
- If GLB forms precipitate, please warm it to 56°C until the precipitate has fully dissolved.

Process

- Add 25µl RJ-Protease to a 1.5ml clean microcentrifuge tube. Add 200µl buffy coat to the tube. Then add 200µl GLB. Pulse vortex for 15 s and incubate at 56 °C for 12min.

  Optional: This protocol is specialized for DNA isolation. For usual and routine applications RNase treatment isn't needed. If RNA-free genomic DNA is required, add 10µl Prime-RNase A, mix by vortexing, and incubate for 5min at room temperature before going to step 2.
- Add 200µl ethanol (%96-100) to the lysate, mix by pulse vortexing for 15 s, then centrifuge briefly.
- Gently, pipette the mixture to a HiPure DR column placed in a 2ml collection tube (supplied in the kit box). Centrifuge at 13000 rpm for 1min. Discard flow-through and place the HiPure DR column in new collection tube (provided in the kit box).

  Note: If the lysate did not pass the column, centrifuge at full speed until it passes through the column.
- Add 600µl BWB1 and centrifuge for 1min at 13000 rpm, discard the flow-through and place the HiPure DR column in the previous collection tube.
- Add 600µl BWB2 and centrifuge for 3min at 14000 rpm. Discard both the flow-through and the collection tube. Place the HiPure DR column in a new clean 1.5ml microcentrifuge tube (not provided).

  Note: To avoid ethanol carry over, be careful that the column does not come into contact with the flow-through, if it happens discard the flow-through, place the column back in a collection tube and centrifuge for another 1min at 14000 rpm.

- Pipette 50-200µl ERR directly onto HiPure DR column. Incubate at 56 ˚C for 3-5min. Centrifuge it at 13000 rpm for 1min.

  Note: If the expected DNA yield is more than the yield from pervious step, put the HiPure DR column on a new microtube and add another 50-200µl ERR, incubate for 5min at 56 ˚C. Then, centrifuge for 1min at 13000 rpm. However, it is possible to pass the follow-through from step 6 once more to obtain DNA with higher concentrations.

Protocol 5: Isolation of Genomic DNA (Animal tissues)

Sample type: Animal tissues (fresh and frozen)

Some tips to know

- All centrifugation steps are carried out at room temperature (15–25°C) in a microcentrifuge.
- If TLB or GLB forms precipitate, please warm it to 56°C until the precipitate has fully dissolved. This is due to storage condition and won't influence the efficiency of the buffer.
- Do not forget to add the appropriate amount of ethanol (96–100%) to BWB1 and BWB2 as indicated on the bottle, before using for the first time, refer to washing buffer preparation part.
- For frozen samples, equilibrate them to room temperature. Avoid repeated freezing and thawing of samples which might lead to a decrease in DNA size.

Process

- Select one of the sample preparation methods here (a, b or c) then Place the prepared sample in a 1.5ml microcentrifuge tube.
- Cut the tissue into small pieces. Then, Place the sample in to a clean microcentrifuge tube.
- Use Micropestle alternatively homogenizer to grind the tissue in TLB Buffer before addition of RJ-Protease.
- Ground the samples under liquid nitrogen (recommended for samples which are difficult to lyse).
- Add 180µl TLB and then add 25µl RJ-Protease to the sample. Mix thoroughly by pulse vortexing for 30 s and then incubate at 56°C for 1-2 h until the tissue is completely lysed. Pulse vortex every 10min during incubation to intersperse the sample, or place it in a thermomixer or shaking water bath.

  Optional: This protocol is specialized for DNA isolation. For usual and routine applications RNase treatment isn't needed. If RNA-free genomic DNA is required, add 10µl Prime-RNase A, mix by vortexing, and incubate for 5min at room temperature before going to step 3.
- Add 200µl GLB to the mixture, mix by pulse vortexing for 10 s and then incubate at 70°C for 10min.
- Add 200µl absolute ethanol, then mix thoroughly by pulse vortexing for 15s.
- Pipette the mixture from step 4 to a HiPure DR column placed in a 2ml collection tube (supplied in the kit box). Centrifuge at 13000 rpm for 1min. Discard flow-through and place the HiPure DR column in the collection tube again.

  Note: If the lysate did not pass the column, centrifuge at full speed until it passes through the column.
- Add 700µl BWB1 and centrifuge for 1min at 13000 rpm, just discard the flow-through. Place the HiPure DR column in the previous collection tube and go to the next step.

- Add 600µl BWB2 and centrifuge for 3min at 14000 rpm. Discard both the flow-through and the collection tube. Place the HiPure DR column in a new clean 1.5ml microcentrifuge tube (not provided).

  Note: To avoid ethanol carry over, be careful that the column does not come into contact with the flow-through, if it happens discard the flow-through, place the column back in a collection tube and centrifuge for another 1min at 14000 rpm.
- Pipette 50-200µl ERR directly onto HiPure DR column. Incubate at room temperature for 1-5min. Centrifuge it at 12000 rpm for 1min.

  Note: If the expected DNA yield is more than the yield from pervious step, put the HiPure DR column on a new microtube and add another 50-200µl ERR, incubate for 5min at room temperature. Then, centrifuge for 1min at 12000 rpm. However, it is possible to pass the follow-through from step 8 once more to obtain DNA with higher concentrations.

Protocol 6: Isolation of Genomic DNA (Bacteria, gram negative)

Sample type: Bacteria (gram negative)

Some tips to know

- All centrifugation steps are carried out at room temperature (15–25°C) in a microcentrifuge.
- If TLB or GLB forms precipitate, please warm it to 56°C until the precipitate has fully dissolved. This is due to storage condition and won't influence the efficiency of the buffer.
- Do not forget to add the appropriate amount of ethanol (96–100%) to BWB1 and BWB2 as indicated on the bottle, before using for the first time, refer to washing buffer preparation part.
- For frozen samples, equilibrate them to room temperature. Avoid repeated freezing and thawing of samples which might lead to a decrease in DNA size.

Process

- Calculate the bacteria cell number (refer to appendix 6, in main handbook). Collect the cell by centrifugation at 10000 rpm for 10min. Discard the supernatant.
- Add 180µl TLB and then 25µl RJ-Protease. Mix thoroughly by pulse vortexing for 30 s, then incubate at 56°C for 1-2 h until the lysate becomes clear. Pulse vortex every 10min during incubation to intersperse the sample, or place it in a thermomixer or shaking water bath.

  Optional: This protocol is specialized for DNA isolation. For usual and routine applications RNase treatment isn't needed. If RNA-free genomic DNA is required, add 10µl Prime-RNase A, mix by vortexing, and incubate for 5min at room temperature before going to step 3.
- Add 200µl GLB, mix by pulse vortexing for 10 s, then incubate at 70°C for 10min.
- Add 200µl absolute ethanol, then mix thoroughly by pulse vortexing for 15 s.
- Pipette the mixture from step 4 to a HiPure DR column placed in a 2ml collection tube (supplied in the kit box). Centrifuge at 13000 rpm for 1min. Discard flow-through and place the HiPure DR column in the collection tube again.

  Note: If the lysate did not pass the column, centrifuge at full speed until it passes through the column.
- Add 700µl BWB1 and centrifuge for 1min at 13000 rpm, discard the flow-through. Place the HiPure DR column in the previous collection tube and go to the next step.

- Add 600µl BWB2 and centrifuge for 3min at 14000 rpm. Discard both the flow-through and the collection tube. Place the HiPure DR column in a new clean 1.5ml microcentrifuge tube (not provided).

  Note: To avoid ethanol carry over, be careful that the column does not come into contact with the flow-through, if it happens discard the flow-through, place the column back in a collection tube and centrifuge for another 1min at 14000 rpm.
- Pipette 50-200µl ERR directly into HiPure DR column. Incubate at room temperature for 1-5min. Centrifuge it at 12000 rpm for 1min.

  Note: If the expected DNA yield is more than the yield from pervious step, put the HiPure DR column on a new microtube and add another 50-200µl ERR, incubate for 5min at room temperature. Then, centrifuge for 1min at 12000 rpm. However, it is possible to pass the follow-through from step 8 once more to obtain DNA with higher concentrations.

Protocol 7: Isolation of Genomic DNA (Bacteria, gram positive)

Sample type: Bacteria (gram positive)

Some tips to know

- All centrifugation steps are carried out at room temperature (15–25°C) in a microcentrifuge.
- For gram positive bacteria like *B. subtilis*, Lysozyme (Cat No. EB983017) should be ordered separately.
- Prepare the lysis buffer as follows:
  - 20 mM Tris.HCl, pH 8.0
  - 2 mM sodium EDTA
  - 1.2% Triton® X-100

    Add lysozyme to 20 mg/ml (immediately before use).
- Preheat a heat block or water bath to 37 °C.
- If TLB or GLB forms precipitate, please warm them to 56°C until the precipitate has fully dissolved. This is due to storage condition and won't influence the efficiency of the buffer.
- Do not forget to add the appropriate amount of ethanol (96–100%) to BWB1 and BWB2 as indicated on the bottle, before using for the first time, refer to washing buffer preparation part.
- For frozen samples, equilibrate them to room temperature. Avoid repeated freezing and thawing of samples which might lead to a decrease in DNA size.

Process

- Calculate the bacteria cell number (refer to appendix 6, in main handbook). Collect the cell by centrifugation at 10000 rpm for 10min. Discard the supernatant.
- Resuspend the pellet, add 180µl enzymatic lysis buffer. Incubate 30-60min at 37 °C.
- Add 200µl GLB and then 25µl RJ-Protease. Mix thoroughly by pulse vortexing for 30 s and then incubate at 56 °C for 30-60min until the sample is completely lysed. Pulse vortex every 10min during incubation to intersperse the sample, or place it in a thermomixer or shaking water bath.

  Optional: This protocol is specialized for DNA isolation. For usual and routine applications RNase treatment isn't needed. If RNA-free genomic DNA is required, add 10µl Prime-RNase A, mix by vortexing, and incubate for 5min at room temperature before going to step 4.
- Centrifuge at 15000 rpm for 2min. Pour supernatant to clean tube.
- Add 200µl absolute ethanol, then mix thoroughly by pulse vortexing for 15 s.

- Pipette the mixture from step 5 to a HiPure DR column placed in a 2ml collection tube (supplied in the kit box). Centrifuge at 13000 rpm for 1min. Discard flow-through and place the HiPure DR column in the collection tube again.

  Note: If the lysate did not pass the column, centrifuge at full speed until it passes through the column.
- Add 700µl BWB1 and centrifuge for 1min at 13000 rpm, just discard the flow-through. Place the HiPure DR column in the previous collection tube and go to the next step.
- Add 600µl BWB2 and centrifuge for 3 min at 14000 rpm. Discard both the flow-through and the collection tube. Place the HiPure DR column in a new clean 1.5ml microcentrifuge tube (not provided).

  Note: To avoid ethanol carry over, be careful that the column does not come into contact with the flow-through, if it happens discard the flow-through, place the column back in a collection tube and centrifuge for another 1min at 14000 rpm.
- Pipette 50-200µl ERR directly into HiPure DR column. Incubate at room temperature for 1-5min. Centrifuge it at 12000 rpm for 1min.

  Note: If the expected DNA yield is more than the yield from pervious step, put the HiPure DR column on a new microtube and add another 50-200µl RRB, incubate for 5min at room temperature. Then, centrifuge for 1min at 12000 rpm. However, it is possible to pass the follow-through from step 9 once more to obtain DNA with higher concentration.

Troubleshooting

For troubleshooting assistance with the DNall VirAll Kit, please consult the following guidelines. The Technical Support Team of Carbon Technologies is available to address any further questions or concerns you may have.

	Symptoms	Problem	Suggestion
Viral DNA Isolation	Low DNA yield	Insufficient lysis	Please refer to Table 3 to apply best match for size of starting material and amount of lysis buffer.
			Make sure to do pulse-vortexing after addition of lysis buffer and RJ-Protease.
		Too few viruses in the sample	Do the test with new samples.
		Incomplete lysing	Repeat the reaction once more and make sure to mix the sample and lysis buffer completely by pulse-vortexing.
		Reagents not applied correctly	Prepare buffers according to the protocol.
			Make sure ethanol is added to BWB1 and BWB2.
			Repeat the procedure with a new sample.
		DNA improperly eluted	The best buffer for DNA rehydration is prepared in the Kit Box. We insist to use the supplied rehydration buffer, however if you want to use water instead, make sure that the pH is at least 7.0, or use 10 mM Tris-HCl Ph≥ 7.0.
	DNA does not perform well in downstream applications	DNA was not washed with the provided washing buffer	Ensure the column was washed once with prepared BWB1 and once more with prepared BWB2, respectively.
		Ethanol carryover	Ensure that the traces of ethanol before rehydration step is removed.
General Handling	Column clogging	Precipitates were not removed.	When using plasma samples, remove visible Cryoprecipitates by centrifugation for 5min at 3000 × g
		Lysate not completely passed through the membrane	Centrifuge for 1min at full speed or until all the lysate has passed through the membrane.

Technical Assistance

For technical assistance, Carbon Technologies ensures your complete satisfaction. Our technical support team comprises highly trained and experienced technologists adept at troubleshooting most problems you may encounter. They can offer expert advice to help you select the most suitable product for your needs. You can contact our technical support team anytime through the following methods:

- Phone: +968-97058350
- Directly submit your questions to the Carbon Technologies technical support team through our website: www.carbontechnologiesco.com
- Email your questions to: technicalsupport@carbontechnologiesco.com

Rest assured, our team is dedicated to providing prompt and effective assistance to address any inquiries or issues you may have. We are committed to ensuring your satisfaction and success in using our products.

Appendix 1: Handling RNA

RNA is highly sensitive to degradation due to the ubiquitous presence of RNases in the environment. unlike DNases, which require metal ions for activity, RNases do not require cofactors for their enzymatic activity and can maintain activity even after prolonged boiling or autoclaving. Since RNases are active enzymes, and are too hard to be deactivated, do not use any plasticware or glassware without first removing possible RNase pollution. Special precautions should be taken when working with RNA. All reagents and equipment must be specially treated to inactivate RNases prior to use. The following are some tips and techniques to remember when working with RNA:

General Tips

- Always wear latex or vinyl gloves while handling reagents and RNA samples to prevent RNase contamination from the surface of the skin or from dusty laboratory mobilizations. These materials are generally RNase-free and do not require pretreatment to inactivate RNase.

- Treat surfaces of benches with commercially available RNase inactivating agents such as RNZO (Order by cat No RN983018 and RN983019).

- Treat non-disposable glassware and plasticware before use, to ensure that it is RNase-free. Thoroughly rinse plasticware with 0.1N NaOH/1mM EDTA, and RNase-free water.

- Use disposable RNase-free plasticware to decrease the possibility of sample contamination.

- Keep purified RNA on ice when aliquots are pipetted for downstream applications.

- Glassware should be immersed in freshly prepared 0.1% (v/v) DEPC in water or ethanol for several hours, followed by draining and autoclaving.

Appendix 2: RNA Storage Condition

RNA samples are commonly stored at −20 °C or −80 °C, eluted in RNase-free water. Under these conditions, RNA is not degraded after 1 year.

RNA Quality

The ratio of absorbance at 260 nm and 280 nm is used to assess the purity of RNA using UV absorption, by a spectrophotometer or Nanodrop. For pure RNA, A260/A280 ratios should be around 2.1. If the ratio is noticeably lower in either case, it may indicate the presence of protein or other contaminants that absorb strongly at or near 280 nm. High A260/A280 purity ratios are not indicative of an issue.

Appendix 3: RNA Integrity

The integrity of RNA molecules is important for experiments that try to reflect the snapshot of gene expression of RNA isolation. The least expensive method for checking RNA integrity is to run the RNA on a 1% DGE agarose gel and examine the ribosomal RNA (rRNA) bands. The upper ribosomal band (28S in eukaryotic cells and 23S in bacterial cells) should be about twice the intensity of the lower band (18S in eukaryotic cells and 16S in bacterial cells) and should be sharp and tight.

Denaturing agarose gel electrophoresis

Formaldehyde is used to keep the RNA from denaturation, and when RNA is heated in the presence of formaldehyde all secondary structures are eliminated. Therefore, gel electrophoresis of RNA in agarose gels containing formaldehyde provides a good denaturing gel system. Formaldehyde is a potential carcinogen. Take appropriate safety measures and wear gloves when handling.

Gel preparation

- Prepare %1.2-2 gel with TAE 1x buffer (%5 formaldehyde).
- Pour the gel using a comb that will form wells large enough to accommodate at least 20µl.
- Assemble the gel in the tank.
- Use TAE 1x buffer (%5 formaldehyde) as the tank buffer.

RNA Sample preparation

- Add 10µg of purified RNA to 6µl of Carbon Technologies sample buffer (in RNA Loading Set, order by cat No LD983007) and incubate at 70 °C for 3min.
- Add 3µl Hinari to the mixture of RNA and sample buffer and mix well by pipetting.
- Then run the mixture in the prepared gel.

Electrophoresis

Load the gel and electrophorese at 5-6 V/cm until the loading dye at least has migrated 2/3 the length of gel.

Results

Visualize the gel on a UV transilluminator. The 28S and 18S ribosomal bands should be sharp and intense. The intensity of the 28S band should be twice more than the 18S band. The following Figure shows different RNA samples, isolated by RNJia kit and run by DGE.

Note: If DNA contamination is present, the high molecular weight smear will be appeared above the 28S band.

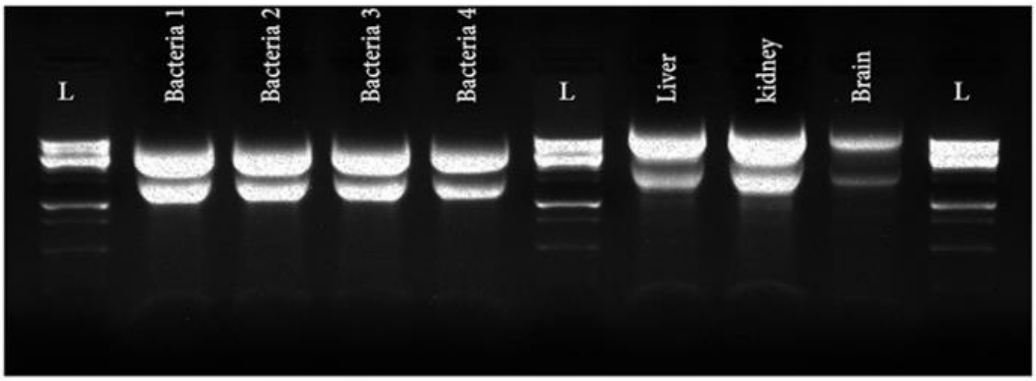

Figure1. Isolated RNA using RNJia Kit, RNA was isolated from the indicated animal tissues and bacteria cells using the RNJia Kit. For each sample, 10 µg RNA was pretreated by Carbon Technologies Sample Buffer and analyzed on Denaturing Gel Electrophoresis (DGE).

Appendix 4: DNA Contamination

Different methods exist for monitoring genomic DNA contamination, for example, using OD measurement or via agarose gel electrophoresis. Nevertheless, genomic DNA traces which are not detectable by the above methods, can produce nonspecific results in sensitive downstream applications. The co-amplification of cDNA and contaminating genomic DNA produce one or more additional PCR fragments that can be traced via agarose gel electrophoresis. strategies to solve the problem:

- Design PCR primers for span exon boundaries, which includes one or more intronic sequence motifs in the genomic DNA, results in amplification of cDNA template.
- Design a minus control reaction with reverse transcriptase. In that specific reaction, instead of the reverse transcriptase enzyme, water is added to the reaction mixture. PCR product in this control reaction indicate genomic DNA contamination.

Appendix 5: Convert RPM to RCF (centrifuge)

All centrifugation steps are performed at room temperature. The correct rpm can be calculated using the formula:

$$RPM = \sqrt{\frac{RCF}{(1.118 \times 10^{-5})(r)}}$$

Where **RCF** = required gravitational acceleration (relative centrifugal force in units of g); **r** = radius of the rotor in cm; and **RPM** = the number of revolutions per minute required to achieve the necessary g-force.

Appendix 6: Cell Count by a Hemocytometer

A: Sample preparation

First resuspend the cell in flask, then quickly remove your aliquot of cells. For a cell culture growing in a Petri dish, you need to resuspend the cells growing on the bottom of the dish by gently using a pipette to remove cells and media from a dish and then gently expelling them back into the dish. This aspiration of the contents of the Petri dish should be completed several times, each time expelling the cells and media while moving the pipette across the bottom of the dish to gently discharge cells growing on the entire surface. After resuspension, the aliquot is quickly removed from the vessel before the cells settle to the bottom again. For cell culture applications cells are often stained with Trypan Blue so that dead cells can be distinguished from live cells. For example, a 0.5 ml suspension of cells would be removed from the Petri dish and mixed with 0.5 ml Trypan Blue solution in an Eppendorf or small test tube. Trypan Blue is a stain that selectively stains dead cells.

B: Loading sample

Load your sample quickly and smoothly. It is important to handle the hemocytometer and the cover slip carefully. Never place your fingers on the reflective surface of the slide. Always clean the slide before you load the sample by rinsing the slide and cover slip with 70-95% Ethanol. Air dry or gently wipe the slide and cover slip with lens paper. Place the clean and dry slide on your work surface and place the coverslip on top to cover the reflective surfaces. If you have diluted your cells in a test tube, invert the tube several times to resuspend the cells. Using a micropipette, quickly and smoothly without interruption, add 10 µl of your cell suspension (or 1 drop from a transfer pipette) to the V-shaped groove on each side of the hemocytometer. If your sample moves into the gutters, you may not have loaded the sample in the correct location or you may have used too large of an aliquot.

C: Estimating cell density

count all of cells within each of the four large quadrants in the four corners of each counting chamber on the hemocytometer (see figure below). Count all of the cells within each quadrant except those on the far-right edge and lower bottom edge.

Calculate the cell density by this formula:

Average number of cells × dilution factor× 10^4

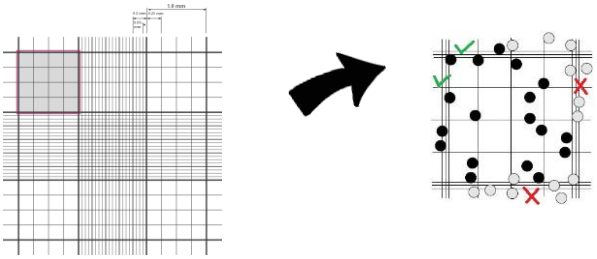

Figure1. Cell counting with hemocytometer

Symbols

Manufacturer

	Date of manufacture
	Use-by date
	Contains sufficient for <n> tests
	Consult instructions for use
	Biological risks
	Temperature limit
REF	Catalogue number
LOT	Lot number

Contact Information

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After-sale Service Center: Carbon Technologies LLC

Release Date: ... ... Date of Manufacture: ... ...