DNall Plus Kit
DNA Isolation Based on Silica Technology

For DNA Isolation from
Blood
Buffy Coat
Animal Tissue
Cultured Cells
Bacteria
Body Fluid
Serum
Plasma
CVS (Chorionic Villus Sampling)
Buccal Cells
Insects
Hair
Rodent Tail
Ear Punches
Amniotic Fluid

Contents

Kit Content

Component	100 Preps, PR242002
TLB	18ml
GLB	25ml
BWB1 (concentrate)	2 x 16ml
BWB2 (concentrate)	2 x 16ml
RRB	20ml
RJ-Protease	2 X 1.25ml
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 suggested storing RJ-Protease at -20°C for longer stability; However, for routine use, it is recommended that RJ-Protease aliquoted to 100µl volumes before storage at 2-8°C 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 Plus Kit provides the components and procedures necessary for purifying genomic DNA from blood, buffy coat, body fluid, buccal cells, animal tissue, serum and plasma, fixed tissue, cultured cells and bacteria. Notice that, DNall Plus Kit is intended for molecular biology applications not for diagnostic use. We recommend all users to study DNA 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 team 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 Plus 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 REF and Lot number on web at www.carbontechnoloesco.com.

Description

DNall Plus Kit provides a rapid, careful, convenient method for high quality genomic DNA isolation from various sample types including blood, buffy coat, body fluid, buccal cells, animal tissue, serum and plasma, fixed tissue,

cultured cells and bacteria. This kit is based on spin column technology for isolation of concentrated, highly purified, intact genomic DNA which is suitable to use for variety of downstream processes such as PCR analysis, restriction endonuclease digestions, Southern blot, genotyping and etc.

Procedure

DNall Plus Kit is designed for isolating DNA from various sample types. Lysis is achieved by incubation of the sample in a RJ-Protease enzyme solution and Carbon Technologies specific lysis buffer. Appropriate conditions for DNA binding to the silica membrane is achieved by the addition of chaotropic salts and ethanol to the lysate. Then, DNA is selectively bound to the membrane. Contaminants are removed by two specific washing buffers. Pure genomic DNA is finally eluted in rehydration buffer. Isolated DNA is ready to use in downstream applications. It has A 260/ A 280 ratios of 1.7–2, and a symmetric peak at 260 nm by spectrophotometer, confirms high purity.

Equipment & Reagents Required But Not Supplied

Ethanol (96%-100)
Pipets and pipet tips
1.5ml Microtube
Vortex
Centrifuge
Micro centrifuge
Dry Heat Block/ Water Bath
Applications
The isolated DNA can be used in many downstream applications:
Different kinds of PCRs, including Long-range PCR
Sequencing
Restriction digestion
Southern blotting
Cloning
Features

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

Table 1: DNall Plus Kit features and specifications

Features	Specifications
Elution volume	50-200µl
Technology	Silica technology
Main sample type	Fresh whole blood/ buffy coat/ body fluids/ serum and plasma/ animal tissue/ cultured cells/ bacteria
Processing	Manual
Sample amount	10-25 mg of tissue Up to 2×109 bacteria cells Up to 5 X 106 cultured cells Up to 200µl blood, buffy coat, body fluid
Operation time per reaction	Less than 20Min (for whole blood, buffy coat, serum and plasma) Less than 2 h (for animal tissue and bacteria)

Typical yield	Varies depending on sample type
Average purity	A260/A280= 1.7-2.0
Size of DNA purified	≈ 50 Kb
Enzyme	RJ-Protease

Recommended Starting Material

To reach optimized results it is better to follow as listed here. The size of recommended material is written in Table 2.

Table 2: Appropriate size of starting material

Sample	Size of Starting Material
Blood*	Up to 250µl
Buffy coat	Up to 200µl
Animal tissue	All tissue kind expect spleen: up to 25 mg (Spleen up to 10mg)
Bacteria cells	2.5 x 10 8 to 2 x 109
Cultured cell	≤5 x 106 cells

*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.

Sample Preparation

Blood

- It is recommended to collect blood in standard collection tube with EDTA as anticoagulants. Other anticoagulants such as ACD (citrate) and heparin may be used. Notice, Heparin has been shown to inhibit Taq polymerase activity and it is recommended to be avoided, when possible.
- For optimum results, do not store sample at 4 °C for more than 5 days.
- Samples stored at -75°C could be used for at least 2 years. Before use, thaw quickly in a 37 °C water bath and keep sample on ice until use.

Buffy Coat

Buffy coat is a leukocyte-enriched fragment of whole blood. Buffy coat contains the majority of the white blood cells and platelets as well as an equivalent amount of genomic DNA (gDNA), when compared to whole blood. For collecting buffy coat is recommended to do as follows:

- Use fresh blood that was collected in standard collection tubes with EDTA as anticoagulants.
- Centrifuge 2.5ml of whole blood for 10minutes at 2,500g.
- Remove upper plasma portion and carefully collect the cells at the interface by using a pipette and place in a separate tube (An approximately tenfold concentration of cells is obtained using this technique 200µl buffy coat from 2.5ml blood).

Animal Tissue

To avoid less DNA quality and quantity, remember to start with fresh samples. Best storage condition for tissue is at –

20°C or –70°C. Avoid freezing and thawing of samples, which results in reduced size of DNA.
All following three ways are possible to use for sample homogenizing:

- Cutting considered tissue into small pieces. Then, transfer the sample in to a clean microcentrifuge tube.
- Using Micropestle alternatively homogenizer or syringe needle to grind the tissue in TLB before addition of RJ-Protease.
- Grounding samples under liquid nitrogen (recommended for samples which are difficult to lyse).

Cultured Cells

Storage: Fresh or frozen samples may be used by DNall Plus Kit. Frozen samples can be kept at -80°C for long time. As a guide, storage preparation stock and conditions are written here.

Cell selection: First, ensure that the cells are in their best possible condition. Select cultures near the end of log phase growth (approximately 90% confluent) and change their medium 24 hours prior to harvesting. Carefully examine the culture for signs of microbial contamination. Facilitate this by growing cultures in antibiotic-free medium for several passages prior to testing. This allows time for any hidden, resistant contaminants (present in very low numbers) to reach a higher, more easily detected level. Samples of these cultures are then examined microscopically and tested by direct culture for the presence of bacteria, yeasts, fungi, and mycoplasmas.

Cell harvesting: Remove all dissociating agents by washing or inactivation (especially important when using serum-free medium). Centrifugation, when absolutely necessary, should only be hard enough to obtain a soft pellet; 100 x g for 5 to 6minutes is usually sufficient. Count and then dilute or concentrate the harvested cell suspension to twice the desired final concentration, which is usually 4 to 10 million viable cells per milliliter. An equal volume of medium containing the cryoprotective agent at twice its final concentration will be added later to achieve the desired inoculum. Keep the cells chilled to slow their metabolism and prevent cell clumping. Avoid alkaline pH shifts by gassing with CO2 when necessary.

Cryoprotection: Cryoprotective agents are necessary to minimize or prevent the damage associated with slow freezing. DMSO is most often used at a final concentration of 5 to 15% (v/v). Always use reagent or other high purity grades that have been tested for suitability. Sterilize by filtration through a 0.2-micron nylon membrane in a polypropylene or stainless-steel housing and store in small quantities (5ml). Some cell lines are adversely affected by prolonged contact with DMSO. This can be reduced or eliminated by adding the DMSO to the cell suspension at 4°C and removing it immediately upon thawing. If this does not help, lower the concentration or try glycerol or another cryoprotectant. Glycerol is generally used at a final concentration of between 5 and 20% (v/v). Sterilize by autoclaving for 15 minutes in small volumes (5ml) and refrigerate in the dark. Although less toxic to cells than DMSO, glycerol frequently causes osmotic problems, especially after thawing. Always add it at room temperature or above and remove slowly by dilution. High serum concentrations may also help cells survive freezing. Replacing standard media-cryoprotectant mixtures with 95% serum and 5% DMSO may be superior for some overly sensitive cell lines, especially hybridomas. Add cryoprotective agents to culture medium (without cells) immediately prior to use to obtain twice the desired final concentration (2X). Mix this 2X solution with an equal volume of the harvested cell suspension to obtain the inoculum for freezing. This method is less stressful for cells, especially when using DMSO as the cryoprotectant.

Cooling rate: The cooling rate used to freeze cultures must be just slow enough to allow the cells time to dehydrate, but fast enough to prevent excessive dehydration damage. A cooling rate of -1°C to -3°C per minute is satisfactory for most animal cell cultures. Larger cells, or cells having less permeable membranes may require a slower freezing rate since their dehydration will take longer. Transfer from the cooling chamber or device to the final storage location must be done quickly to avoid warming of the vials. Use an insulated container filled with dry ice or liquid nitrogen as a transfer vessel to ensure that the cells remain below -70°C.

Thawing: Remove the vial from its storage location and carefully place the vessel in warm water, agitate gently until completely thawed. Rapid thawing (60 to 90 seconds at 37°C) provides the best recovery for most cell cultures; it reduces or prevents the formation of damaging ice crystals within cells during rehydration.

Recovery: Since some cryoprotective agents may damage cells upon prolonged exposure, remove the agents as quickly and gently as possible. Several approaches are used depending on both the cryoprotective agents and characteristics of the cells. Most cells recover normally if they have the cryoprotective agent removed by a medium change within 6 to 8 hours of thawing. Transfer the contents of the vial to a T-75 flask or other suitable vessel containing 15 to 20 milliliters of culture medium and incubate normally. As soon as a majority of the cells have attached, remove the medium containing the now diluted cryoprotective agent and replace with fresh medium.

For cells that are sensitive to cryoprotective agents, removing the old medium is easily accomplished by gentle centrifugation. Transfer the contents of the vial to a 15ml centrifuge tube containing 10ml of fresh medium and spin for 5minutes at 100 x g. Discard the supernatant containing the cryoprotectant and resuspend the cell pellet in fresh medium. Then transfer the cell suspension to a suitable culture vessel and incubate normally.

When glycerol is used as the cryoprotectant, the sudden addition of a large volume of fresh medium to the thawed cell suspension can cause osmotic shock, damaging or destroying the cells. Use several stepwise dilutions with an equal volume of warm medium every 10minutes before further processing to give the cells time to readjust their osmotic equilibrium.

Preparation: It is crucial to use the correct amount of starting material. DNA content can vary greatly from cell to cell. So, counting cells is the most important step before starting the procedure (for more information refer to appendix 3). However, as a guide, the number of HeLa cells after confluent growth obtained in various culture vessels, is given in Table 3. After counting and selecting the intended cell volume, continue the procedure with appropriate protocol.

Table 3: Number of HeLa Cells in various culture vessels

Vessel Type	Size	Cell Number
Dishes	35mm	1 x 106
	60 mm	2.5 x 106
	100 mm	7 x 106
	145-150 mm	2 x 107
Flask	40-45ml	3 x 106
	250-300ml	1 x 107
	650-750ml	2 x 107
Multiwell-plates	96-wells	4-5 x 104
	48-wells	1 x 105

	24-wells	2.5 x 105
	12-wells	5 x 105
	6-wells	1 x 106

Bacteria

Typical yields of DNA will vary depending on the cell density of the bacterial culture and the bacterial species, hence before starting, it's recommended to determine your bacterial species. As a guide, bacteria culture preparation and storage conditions are written here.

Storage: Fresh or frozen bacteria samples may be used by DNall Plus Kit. Frozen samples can be kept at -80°C for long time. As a guide, storage preparation stock and condition are written here.

Bacteria culture: The following protocol is for inoculating an overnight culture of liquid LB with bacteria.
Prepare liquid Luria-Bertani (LB)
To make 400ml of LB, weigh out the following into a 500ml glass bottle:

- 4 g NaCl
- 4 g Tryptone
- 2 g Yeast Extract
- and dH2O to 400ml

Loosely close the cap on the bottle and then loosely cover the entire top of the bottle with aluminum foil. Autoclave and allow to cool to room temperature. Now screw on the top of the bottle and store the LB at room temperature.

- Use a single, well-isolated colony from a fresh Luria-Bertani (LB) agar plate to inoculate 1–10ml of LB medium.
- Loosely cover the culture with sterile aluminum foil or a cap that is not airtight.
- Incubate bacterial culture at 37°C for 12-18 hour in a shaking incubator

  Note: Incubation time can be optimized to increase the DNA yield for a given culture volume. However, it has been observed that as a culture ages the DNA yield may begin to decrease due to cell death and lysis within the culture.

  Note: For DNA isolation, bacteria should be harvested in log-phase growth.

Storing condition

- Autoclave microcentrifuge tube or 1-3ml screw cap.
- Grow a fresh overnight culture of the strain in broth. Do not grow the cultures too long. Bacteria strains should be grown to late log phase.
- Label the tube with the strain and date.
- Either 5% to 10% DMSO or glycerol can be used as cryopreservation in the culture medium. Glycerol is usually prepared in aqueous solution at double the desired final concentration for freezing. It is then mixed with an equal amount of cell suspension.
- Aliquot 1 to 1.8ml of bacteria to each vial and seal tightly with screw cap.
- Allow the cells to equilibrate in the freeze medium at room temperature for a minimum of 15 min but no longer than 40 min. After 40 min, the viability may decline if DMSO is used as the cryoprotectant.
- Place the vials into a pre-cooled (4°C), controlled rate freeze chamber and place the chamber in a mechanical freezer at -70°C for at least 24 hours.

- Quickly transfer the vials to liquid nitrogen or at -130°C freezer. After 24 hours at -130°C, remove one vial, restore the bacteria in the culture medium and check viability and sterility.

Recovery of cryopreserved cells

- Prepare a cultured vessel that contains at least 10ml of the appropriate growth medium equilibrate for both temperature and pH.
- Remove the vial containing the strain of interest and thaw by gentle agitation in a 37 °C water bath (or a bath set at the normal growth temperature for that bacterial strain). Thaw the strain rapidly until all ice crystals have been melted (approximately 2min).
- Remove the vial from the bath and decontaminate it by dipping in or spraying with 70% Ethanol. Unscrew the top of the vial and transfer the entire content to the prepared growth medium. Examine the cultures after an appropriate length of time. If the broth shows growth in 1-2 days, streak a plate from the broth and verify that is the correct strain.

Preparation: It is crucial to use the correct amount of starting material. DNA content can vary greatly between different bacteria types. So, counting cells is the most important step before starting the procedure. The input bacterial cell amount should not exceed 2 × 109 cells. For example, for E. coli, depending on culture growth, this is equivalent to 0.5 - 1.0ml of an overnight culture. It is not recommended to exceed 1ml of culture for this procedure. It is important to measure bacterial growth by spectrophotometer before starting the protocol. (For cell counting guideline refer to appendix 3, part B and C). After counting and selecting the intended cell volume, continue the procedure with appropriate protocol.

Before Start

- If GLB or TLB
- To BWB1 and BWB2 as indicated on the bottle, before using for the first time, refer to washing buffer preparation. Forms precipitate, please warm it to 56°C until the precipitate has fully dissolved. This is due to storage condition and will not influence the efficiency of buffer.
- Not forget to add the appropriate amount of ethanol (96%–100)

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 4. 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 4: Washing buffer preparation

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

Maximize DNA Yield

To obtain higher yield of DNA, it is important to follow protocol carefully and pay attention to sample size table (refer to table 2), which is recommended for samples. It is good to know that:

- Yield and quality of the purified DNA depend on sample storage conditions. For best results, it is recommended to use fresh samples, however for long-term storage, it is better to freeze samples immediately and store them at -20°C or -70°C. Blood sample should be stored at room temperature for no longer than 24 hours or at 4°C for no longer than 5 days. For long-term storage, freeze blood at –70°C. Storing blood at -20°C, can compromise the integrity of the sample, then results in reducing yields and quality of DNA.
- Avoid freezing and thawing samples, which may result in decreased DNA yield and size, compared to fresh samples.

Protocols

Protocol 1: Isolation of Genomic DNA (Animal cell, Cells, Body fluid, Serum and plasma)

Sample type:

- Animal Blood
- Cells
- Body Fluid
- Serum
- Plasma

Some tips to know

- All centrifugation steps are carried out at room temperature (15–25°C).
- If RNase treatment is desired, Prime-RNase can be ordered separately from Carbon Technologies, Cat NO EB983013.
- 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 12 min.

  Note: For cell pellets, add 250µl 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 (Cat NO. EB983013), 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 RRB directly onto HiPure DR column. Incubate at room temperature for 1-5min. 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 RRB, incubate for 5min at room temperature. Then, centrifuge for 1 min at 8000 rpm. However, it is possible to pass the follow-through from step 6 once more to obtain DNA with higher concentrations.

Protocol 2: 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).
- If RNase treatment is desired, Prime-RNase A can be ordered separately from Carbon Technologies, Cat No. EB983013.
- 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 (Cat NO. EB983013), 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 RRB 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 RRB, 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 3: 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 RNase treatment is desired, Prime-RNase A, can be ordered separately from Carbon Technologies, Cat No. EB983013.
- 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 (Cat No. EB983013), 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 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, 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 RRB 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 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 8 once more to obtain DNA with higher concentrations.

Protocol 4: 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 RNase treatment is desired, Prime-RNase A, can be ordered separately from Carbon Technologies, Cat No. EB983013.
- 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 3, 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 (Cat No. EB983013), 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 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 RRB 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 8 once more to obtain DNA with higher concentrations.

Protocol 5: 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 RNase treatment is desired, Prime-RNase A, can be ordered separately from Carbon Technologies, Cat No. EB983013.
- 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 3, 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 (Cat No. EB983013), 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 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 RRB 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 concentrations.

Protocol 6: Isolation of Genomic DNA (Amniotic fluid)

Sample type: Amniotic fluid

Some tips to know:

- All centrifugation steps are carried out at room temperature (15–25°C).
- If RNase treatment is desired, Prime-RNase can be ordered separately from Carbon Technologies, Cat NO. EB983013.
- 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.
- If GLB forms precipitate, please warm it to 56°C until the precipitate has fully dissolved.

Process

- Centrifuge 1.5ml of the amniotic fluid for 10min at 5000 rpm in a clean microcentrifuge tube, discard the supernatant and repeat this step until you pellet 5-6ml amniotic fluid.
- Add 200µl GLB to the pellet then 25µl RJ-Protease. Pulse vortex until the pellet is dissolved thoroughly incubate at 56°C for 12min, pulse vortex for 15 s every 6min during the incubation.
- 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 both the flow-through and the collection tube. 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 25-50µl RRB directly onto HiPure DR column. Incubate at room temperature for 5min. Centrifuge it at 12000 rpm for 1min.

Troubleshooting

For troubleshooting assistance with the DNall Plus 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
Low DNA yield	Inappropriate sample storage condition	Please refer to sample preparation guidelines.
	Insufficient lysis	Too much starting material results in low DNA yield. To optimize the results, refer to Table 2.
		Make sure to do pulse-vortexing vigorously after addition of lysis buffer and RJ-Protease.
		Incubate mixture of the sample and lysis buffer for an additional 15-20min at 56° C.
		Ensure mixing sample completely before incubation step.
	Too few cells in the sample	Do the test with new samples.
	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.
	Ethanol from the washing buffer is present in elution	Preform another centrifugation before rehydration step to ensure no remaining of ethanol on column.
	DNA elution is incomplete	Perform rehydration step once more, by adding another 50-200µl rehydration buffer to column and incubate at room temperature before centrifugation. Check that all previous steps are done appropriately.
	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.
Degradation	Sample contaminated with DNase	Be sure to do the process in accordance with the reference protocol.
	The genomic DNA was handled improperly	Reduce vertexing times during mixing steps (not more than recommended).
	Improper sample storage	Please refer to sample preparation guidelines.
	Too old sample	Old samples stored at inappropriate conditions always yield sheared DNA.
Low 260/280 ratio	Sample was diluted in water	It is recommended to use Carbon Technologies rehydration buffer for DNA elution, however if

		you want to use water instead make sure that the pH is at least 7 or use 10 mM Tris-HCl Ph≥ 7.0.
	Protein contamination	This is often due to exceeding the amount of starting material. Follow precisely the respective protocol. If DNA purification is still problematic further reduce the amount of starting material.
High 260/280 ratio	RNA contamination	This kit is optimized to extract DNA without RNA contamination. However, if you need to make sure that no RNA contamination is present, you can purchase Prime-RNase A (Cat No. EB983013) separately and perform RNase treatment during the process.
DNA does not perform well in downstream applications	PCR reaction condition is not optimized	Make sure that PCR condition is optimized by testing: Primer designs and annealing conditions Changing source of Taq Polymerase Different amount of DNA sample
	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	Preform another centrifugation before rehydration step to ensure no remaining of ethanol on column.
	Do not use standard buffer for DNA rehydration	Use Carbon Technologies rehydration buffer for dissolving purified DNA.
Clogged Column	Maximum amount of sample exceeded kit specifications	Refer to specifications to determine if amount of starting material falls within kit specifications.
	The lysate mixture is not homogeneous	To ensure a homogeneous solution, vortex for 10-15 seconds before applying the lysate to the spin column.
	The sample is too much	Use less starting material. The problem can be solved by increasing the g-force and/or centrifuging for a longer period of time until the lysate passes through the column.

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: Yield and Purity of DNA

The absorbance of DNA can be measured by any spectrophotometer. The ratio of absorbance at 260nm and 280nm is used to evaluate the purity of DNA. Pure DNA has an A260/A280 ratio of 1.7–2.0 and also a symmetric peak of absorbance at 260nm. If the ratio is lower in either case, it may indicate the presence of contamination. Proteins have absorbance at 280 nm. EDTA, carbohydrate and phenol all have absorbance near 230nm. Table below shows typical DNA yields from different sample source using DNall Plus Kit.

Table 5: Typical DNA yield from different sample sources

Source	Starting Amount	DNA Yield	DNA Quality (A260/ A280)
Whole blood	250µl	3-12µg	1.7-1.99
Buffy Coat	200µl	15-50µg	1.7-1.99
Cell	Up to 5 x 106	15-50µg	1.7-1.99
Liver	25mg	15-40µg	1.7-1.99
Brain	25mg	8-18µg	1.7-1.99
Kidney	25mg	15-40µg	1.7-1.99
Spleen	10mg	15-40µg	1.7-1.99
Ear	25mg	15-40µg	1.7-1.99
Muscle	25mg	5-10µg	1.7-1.99
Skin	25mg	15-40µg	1.7-1.99
Heart	25mg	10-25µg	1.7-1.99
Lung	25mg	10-25µg	1.7-1.99
Mouse Tail	25mg	15-40µg	1.7-1.99
Rat Tail	25mg	10-40µg	1.7-1.99

Appendix 2: 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 3: 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 0.5ml suspension of cells would

be removed from the Petri dish and mixed with 0.5ml Trypan Blue solution in an Eppendorf or small test tube. Trypan Blue is a stain that selectively stains dead cells.

B: Loading sample

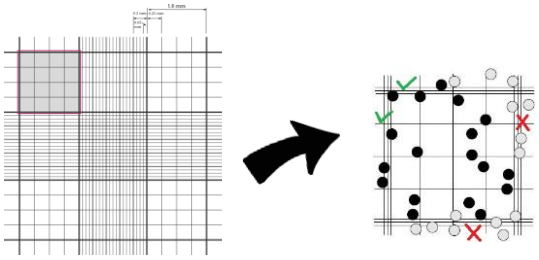
Load your sample quickly and smoothly. It is important to handle the hemocytometer and the cover slip carefully. Never place your fingers on the reflective surface of the slide. Always clean the slide before you load the sample by rinsing the slide and cover slip with 70-95% Ethanol. Air dry or gently 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 10µl 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× 104

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

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After-sale Service Center: Carbon Technologies LLC

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