ROJETECHNOLOGIES

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DNJia Plus Tissue & Bacteria Kit

DNA isolation based on silica technology

- MiniPrep

For DNA Isolation from

Animal tissue
Bacteria
Rodent tails
Ear punches
Insects
Hair

By ROJE

Edition, 2020

ROJETechnologies has been founded since 2014, and manufactures a wide range of molecular biology kits. We research, develop and create our products in order to make easier and more comfortable approaches to do research in molecular biology. Our target is offering high-quality affordable Molecular and diagnostic Kits and reagents, comparable of the world leaders, to research centers, laboratories, clinics, hospitals and diagnostic centers all over the world.

Contents

Kit Content

Component	50 preps	100 preps
TLB	9 ml	18 ml
GLB	10 ml	20 ml
BWB1 (concentrate)	16 ml	2 x 16 ml
BWB2 (concentrate)	16 ml	2 x 16 ml
RRB	10 ml	20 ml
RJ-Protease	1.25 ml	2 X 1.25 ml
HiPure DR Column	50	100
Collection Tube	50	100

Storage

Shipment condition is checked by ROJETechnologies. After arrival, all reagents should be kept dry, at room temperature. We suggested to store RJ-Protease at -20 °C for longer stability; However, for routine use it could be stored at 2-8 °C. When storage condition is as directed, all reagents are stable until expiration date, as indicated on the kit box.

Intended Use

DNJia Plus Tissue & Bacteria Kit provides the components and procedures necessary for purifying genomic DNA from animal tissue and bacteria cells. Notice that, DNJia Plus Tissue & Bacteria 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

ROJETechnologies 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, ROJE team will replace the Kit for you.

Notice to Purchaser

This product is only for experiments and not for commercial use in any kind. No right to resell this kit or any components. For information about our licensing or distributors contact ROJE business team.

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.rojetechnologies.com.

Quality Control

DNJia Plus Tissue & Bacteria Kit is tested against predetermined experiments on a lot-to-lot basis according to ROJETechnologies 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.rojetechnoloes.com.

Description

DNJia Plus Tissue and Bacteria Kit Provides a fast, reliable, meticulous method for genomic DNA isolation from various sample types including animal tissues 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, Southern blot, genotyping and etc.

Procedure

DNJia Plus Tissue and Bacteria Kit is designed for isolating DNA from up to 25 mg tissue sample or up to 2 x 109 bacteria cells. Lysis is achieved by incubation of the sample in a RJ-Protease enzyme solution and TLB. 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 to Be Supplied by User

- Ethanol (96-100%)
- Pipets and pipet tips
- 1.5 ml 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 DNJia Plus Tissue & Bacteria Kit are listed here in Table 1.

Table 1. DNJia Plus Tissue & Bacteria Kit features and specifications

Features	Specifications
Elution volume	50-200μl
Technology	Silica technology
Main sample type	animal tissue/ bacteria
Processing	Manual
Sample amount	• 10-25 mg of tissue • Up to 2×10^9 bacteria cells
Operation time per reaction	Less than 2 h
Typical yield	35 μg
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 (min-max)
Liver	1-25 mg
Brain	1-25 mg
Kidney	1-25 mg
Spleen	1-10 mg
Ear	1-25 mg
Muscle	1-25 mg
Skin	1-25 mg
Heart	1-25 mg
Lung	1-25 mg
Mouse tail	1-25 mg
Rat tail	1-25 mg
Bacteria cells	250 x 10^{8} to 2 x 10^{9}

Sample Preparation

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).

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 DNJia Plus Tissue & Bacteria 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.

1. Prepare liquid Luria-Bertani (LB)

   To make 400 ml of LB, weigh out the following into a 500 ml glass bottle:

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

   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.

2. Use a single, well-isolated colony from a fresh Luria-Bertani (LB) agar plate to inoculate 1–10 ml of LB medium.
3. Loosely cover the culture with sterile aluminum foil or a cap that is not airtight.
4. 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

1. Autoclave microcentrifuge tube or 1-3 ml screw cap.
2. 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.
3. Label the tube with the strain and date.
4. 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.
5. Aliquot 1 to 1.8 ml of bacteria to each vial and seal tightly with screw cap.
6. 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.
7. 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.
8. 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

1. Prepare a cultured vessel that contains at least 10 ml of the appropriate growth medium equilibrate for both temperature and pH.
2. 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 2 min).

3. 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 2×10^9 cells. For example, for E. coli, depending on culture growth, this is equivalent to 0.5 - 1.0 ml of an overnight culture. It is not recommended to exceed 1 ml 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 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%) to BWB1 and BWB2 as indicated on the bottle, before using for the first time, refer to washing buffer preparation.

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 3. 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 3: Washing buffer preparation

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

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 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 ROJETechnologies, 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

1. Select one of the sample preparation methods here (a, b or c) then Place the prepared sample in a 1.5 ml microcentrifuge tube.

    a. Cut the tissue into small pieces. Then, Place the sample in to a clean microcentrifuge tube.
    b. Use Micropestle alternatively homogenizer to grind the tissue in TLB Buffer before addition of RJ-Protease.
    c. Ground the samples under liquid nitrogen (recommended for samples which are difficult to lyse).

2. Add 180 µl TLB and then add 25 µl 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 10 min 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 10 µl Prime-RNase A (Cat No. EB983013), mix by vortexing, and incubate for 5 min at room temperature before going to step 3.

3. Add 200 µl GLB to the mixture, mix by pulse vortexing for 10 s and then incubate at 70°C for 10 min.
4. Add 200 µl absolute ethanol, then mix thoroughly by pulse vortexing for 15 s.
5. Pipette the mixture from step 4 to a HiPure DR column placed in a 2 ml collection tube (supplied in the kit box). Centrifuge at 13000 rpm for 1 min. 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.
6. Add 700 µl BWB1 and centrifuge for 1 min at 13000 rpm, just discard the flow-through. Place the HiPure DR column in the previous collection tube and go to the next step.
7. Add 600 µl 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 1.5 ml 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 1 min at 14000 rpm.
8. Pipette 50-200 µl RRB directly onto HiPure DR column. Incubate at room temperature for 1-5 min. Centrifuge it at 12000 rpm for 1 min.

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 50-200 µl RRB, incubate for 5 min at room temperature. Then, centrifuge for 1 min at 12000 rpm. However, it is possible to pass the follow-through from step 8 once more to obtain DNA with higher concentrations.

Protocol 2: 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 ROJETechnologies, 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

1. Calculate the bacteria cell number (refer to appendix 3, in main handbook). Collect the cell by centrifugation at 10000 rpm for 10 min. Discard the supernatant.
2. Add 180 µl TLB and then 25 µl 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 10 min 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 10 µl Prime-RNase A (Cat No. EB983013), mix by vortexing, and incubate for 5 min at room temperature before going to step 3.

3. Add 200 µl GLB, mix by pulse vortexing for 10 s, then incubate at 70°C for 10 min.
4. Add 200 µl absolute ethanol, then mix thoroughly by pulse vortexing for 15 s.

5. Pipette the mixture from step 4 to a HiPure DR column placed in a 2 ml collection tube (supplied in the kit box). Centrifuge at 13000 rpm for 1 min. 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.

6. Add 700 µl BWB1 and centrifuge for 1 min at 13000 rpm, discard the flow-through. Place the HiPure DR column in the previous collection tube and go to the next step.
7. Add 600 µl 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 1.5 ml 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 1 min at 14000 rpm.

8. Pipette 50-200 µl RRB directly into HiPure DR column. Incubate at room temperature for 1-5 min. Centrifuge it at 12000 rpm for 1 min.

   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 50-200 µl RRB, incubate for 5 min at room temperature. Then, centrifuge for 1 min at 12000 rpm. However, it is possible to pass the follow-through from step 8 once more to obtain DNA with higher concentrations.

Protocol 3: *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 ROJETechnologies, 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

1. Calculate the bacteria cell number (refer to appendix 3, in main handbook). Collect the cell by centrifugation at 10000 rpm for 10 min. Discard the supernatant.
2. Resuspend the pellet, add 180 µl enzymatic lysis buffer. Incubate 30-60 min at 37 °C.

3. Add 200 µl GLB and then 25 µl RJ-Protease. Mix thoroughly by pulse vortexing for 30 s and then incubate at 56 °C for 30-60 min until the sample is completely lysed. Pulse vortex every 10 min 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 10 µl Prime-RNase A (Cat No. EB983013), mix by vortexing, and incubate for 5 min at room temperature before going to step 4.

4. Centrifuge at 15000 rpm for 2 min. Pour supernatant to clean tube.
5. Add 200 µl absolute ethanol, then mix thoroughly by pulse vortexing for 15 s.
6. Pipette the mixture from step 5 to a HiPure DR column placed in a 2 ml collection tube (supplied in the kit box). Centrifuge at 13000 rpm for 1 min. 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.
7. Add 700 µl BWB1 and centrifuge for 1 min at 13000 rpm, just discard the flow-through. Place the HiPure DR column in the previous collection tube and go to the next step.
8. Add 600 µl 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 1.5 ml 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 1 min at 14000 rpm.
9. Pipette 50-200 µl RRB directly into HiPure DR column. Incubate at room temperature for 1-5 min. Centrifuge it at 12000 rpm for 1 min.

   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 50-200 µl RRB, incubate for 5 min at room temperature. Then, centrifuge for 1 min at 12000 rpm. However, it is possible to pass the follow-through from step 9 once more to obtain DNA with higher concentrations

Troubleshooting

Here we try to cover as many problems as you may see in using this product, however scientists in ROJE Technical Support Team are eager to answer all your questions. Do not hesitate to contact us for more information.

Symptoms	Problem	Suggestion
Low yield	Inappropriate sample storage condition	• Best storage condition for tissue is at -20°C or -70°C. Avoid freezing and thawing of samples, which results in reducing size of DNA.
	Incomplete cell lysis	• Too much starting material results in low DNA yield. to optimize the results, refer to Table 2.
	Insufficient mixing of Sample with TLB	• Ensure mixing sample completely before incubation step.
	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 was eluted with water instead of Elution Buffer	• 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 is too old	• Old samples may have degraded DNA in the rehydration step.
	Improper sample storage	• Refer to sample preparation tips.

	The genomic DNA was handled improperly	• Reduce vertexing times during mixing steps (not more than recommended)
Low 260/280 ratio	Insufficient lysis	• Increase incubate time in lysis buffer
	Sample was diluted in water	• It is recommended to use ROJE 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 or on the prepared DNA.
Not performing well in downstream application	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
	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 ROJE Rehydration buffer for dissolving purified DNA.
Clogged Column	Maximum amount of tissue 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 large	• 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

Appendix 1: Yield and Purity of DNA

The absorbance of DNA can be measured by any spectrophotometer. The ratio of absorbance at 260 nm and 280 nm 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 260 nm. 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 230 nm. Table below shows typical DNA yields from different sample source using DNJia Plus Tissue & Bacteria Kit.

Table 4. Typical DNA yield from different sample sources

Source	Starting Amount	DNA Yield	DNA Quality (A260/ A280)
Liver	25 mg	15-40 μg	1.7-1.99
Brain	25 mg	8-18 μg	1.7-1.99
Kidney	25 mg	15-40 μg	1.7-1.99
Spleen	10 mg	15-40 μg	1.7-1.99
Ear	25 mg	15-40 μg	1.7-1.99
Muscle	25 mg	5-10 μg	1.7-1.99
Skin	25 mg	15-40 μg	1.7-1.99
Heart	25 mg	10-25 μg	1.7-1.99
Lung	25 mg	10-25 μg	1.7-1.99
Mouse Tail	25 mg	15-40 μg	1.7-1.99
Rat Tail	25 mg	10-40 μg	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

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.

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

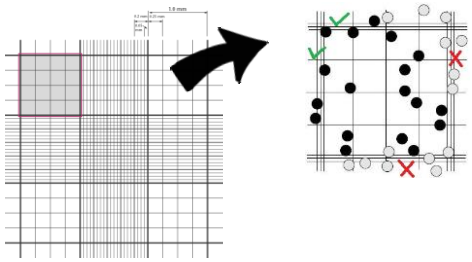

Figure1. Cell counting with hemocytometer

Ordering Information

Category	Product name	Cat NO.	Size
DNA Technologies	DNJia Plus Tissue & Bacteria Kit	DN983050	50 preps
	DNJia Plus Tissue & Bacteria Kit	DN983051	100 preps
Related Products	DNJia Plus Blood & Cell Kit	DN983047	50 preps
	DNJia Plus Blood & Cell Kit	DN983046	100 preps
	DNall Plus Kit	DN983048	50 preps
	DNall Plus Kit	DN983049	100 preps
	DNall Kit	DN983037	50 preps
	DNall Kit	DN983038	100 preps
	DNSol, MiniPrep	DN983002	50 preps
	DNSol, MiniPrep	DN983003	100 preps
	DNSol, MiniPrep	DN983004	200 preps
	DNSol, MidiPrep	DN983014	50 preps
	DNSol, MaxiPrep	DN983018	50 preps
	DNSol Clotted Blood Kit	DN983032	50 preps
	DNJia AmnioPure Kit	DN983044	50 preps
	DNJia AmnioPure Kit	DN983045	100 preps
	DNJia FFPE Tissue Kit	DN983057	50 preps
	DNJia FFPE Tissue Kit	DN983058	100 preps
	Hashin	LD983003	2 ml
	Sor	LD983005	2 ml
	RJ-Protease, Recombinant (20mg/ml)	EB983121	1 ml

Technical Assistance

ROJETechnologies guarantees your complete satisfaction. ROJE technical support team composed of highly trained experienced scientists, who are able to troubleshoot most problems you face. Our technical support team can offer expert advice which may help you select suitable product.

Contact our technical support at any time by selecting one of these ways:

- Through our telephone and fax number available at ROJETechnologies website.
- You can submit your question directly to ROJE Technical Support Team from our website (www.rojetechnologies.com).
- Or send your questions to this email address, technicalsupport@rojetechnologies.com.

ROJETECHNOLOGIES has been founded since 2014, and manufactures a wide range of molecular biology kits. We research, develop and create our products in order to make easier and more comfortable approaches to do research in molecular biology. Our target is offering high-quality affordable molecular and diagnostic Kits and reagents, comparable of the world leaders, to research centers, laboratories, clinics, hospitals and diagnostic centers all over the world.

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