ROJETECHNOLOGIES

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DNJia Plus Blood and Cell Kit

DNA isolation based on silica technology

- MiniPrep

For DNA Isolation from

Blood
Buffy coat
Cultured cells
Body fluid
Serum
Plasma
Buccal cells

By ROJE

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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	100 preps
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 ROJETechnologies. 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. When storage condition is as directed, all reagents are stable until expiration date, as indicated on the kit box.

Intended Use

DNJia Plus Blood and Cell Kit provides the components and procedures necessary for purifying genomic DNA from blood, buffy coat, serum, plasma and cultured cells. Notice that, DNJia Plus Blood and Cell 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 Blood and Cell 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 Blood and Cell Kit provides a rapid, careful, convenient and phenol-free method for high quality genomic DNA isolation from whole blood, buffy coat, body fluid, plasma, serum, animal cultured cell etc. The procedure is based on spin column technology, which takes less than 20 minutes. DNJia Plus Blood and Cell Kit needs less handling and it is convenient for simultaneous isolation, which makes it favorites for laboratory with many isolations in a day. Typical yields of genomic DNA vary, depending on cell density of blood samples. The isolated DNA is ready to use in downstream applications such as PCR analysis and restriction endonuclease digestions.

Procedure

DNJia Plus Blood and Cell Kit is designed for isolating DNA from whole blood, buffy coat, body fluid, plasma, serum, animal cultured cell etc. Lysis is achieved by incubation of the sample in a RJ-Protease enzyme solution and GLB. Appropriate conditions for DNA binding to the silica membrane is achieved by the addition of 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
- 1.5ml 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 DNJia Plus Blood and Cell Kit are listed here in Table 1.

Table 1. DNJia Plus Blood and Cell Kit features and specifications

Features	Specifications
Elution volume	50-200 µl
Technology	Silica technology
Main sample type	Fresh whole blood/buffy coat/ cultured cells/ body fluids/ serum/ plasma
Processing	Manual
Sample amount	Up to 250 µl blood, buffy coat, body fluid, serum, and plasma and up to 5 X 10^6 cultured cells.
Minimum blood input	10 µl
Maximum blood input	250 µl
Operation time per reaction	Less than 20 Min

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

Recommended Starting Material

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

Table 2: Recommended starting material and Lysis Buffer amount

Sample	Size of Starting Material	Lysis Buffer Amount
Blood*	Up to 250 µl	250 µl
Buffy coat	Up to 200 µl	200 µl
Cultured cell	≤5 x 10^6 cells	250 µl

*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 10 minutes at 2,500 g
- 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 200 µl buffy coat from 2.5ml blood).

Cultured Cells

Storage: Fresh or frozen samples may be used by DNJia Plus Blood and Cell 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 6 minutes 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 5 minutes 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 10 minutes 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	Volume	Cell Number
Dishes	35mm	1×10^6
	60mm	2.5×10^6
	100mm	7×10^6
	145-150mm	2×10^7
Flask	40-45ml	3×10^6
	250-300ml	1×10^7
	650-750ml	2×10^7
Multiwell-plates	96-wells	$4\text{-}5 \times 10^4$
	48-wells	1×10^5
	24-wells	2.5×10^5
	12-wells	5×10^5
	6-wells	1×10^6

Before Start

- If GLB 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 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 ROJETechnologies, 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 25 µl RJ-Protease to a 1.5ml clean microcentrifuge tube. Add 250 µl blood (plasma, serum, body fluid and etc.) to the tube. Then add 250 µl 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 10 µl Prime-RNase A (Cat NO. EB983013), mix by vortexing, and incubate for 5min at room temperature before going to step 2.

- Add 250 µl 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 600 µl BWB1 and centrifuge for 1min at 8000 rpm, discard the flow-through and place the HiPure DR column in the previous collection tube.

- Add 600 µl 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 50-200 µl 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 50-200 µl RRB, 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 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 ROJETechnologies, 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 25 µl RJ-Protease to a 1.5ml clean microcentrifuge tube. Add 200 µl buffy coat to the tube. Then add 200 µl 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 10 µl 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 600 µl BWB1 and centrifuge for 1min at 13000 rpm, discard the flow-through and place the HiPure DR column in the previous collection tube.
- Add 600 µl 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 50-200 µl 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 50-200 µl 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.

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 DNA yield	The blood sample was too old	• Please refer to sample preparation guidelines.
	Insufficient lysis	• Please refer to Table 2 to apply best match for size of starting material and amount of lysis buffer.
		• 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.
	Too few white blood cells in the sample	• Do the test with new blood samples.
	Whole blood sample was not mixed before processing	• White blood cells should be in suspension. So, make sure to mix whole blood samples before processing.
	Incomplete lysing of WBC's	• 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.

Degradation	Sample contaminated with DNase	• Be sure to do the process in accordance with the reference protocol.
	Inappropriate sample collection or storage of starting material	• Please refer to sample preparation guidelines.
	Too old sample	• Old samples stored at inappropriate conditions always yield sheared DNA.
Poor DNA Quality	RNA can be copurified with the genomic DNA	• RNase treatment can be performed.
	Incomplete cell lysis	• Incubate sample with lysis buffer and RJ-Protease for an extra 5-10 minutes.
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

Ordering Information

Category	Product name	Cat NO.	Size
	DNJia Plus Blood and Cell Kit	DN983047	100 preps
	DNJia AmnioPure Kit	DN983045	100 preps
	DNSol, MiniPrep	DN983003	100 preps
	DNSol, MidiPrep	DN983014	50 preps
	DNSol, MaxiPrep	DN983018	50 preps
	DNSol Clotted Blood Kit	DN983032	50 preps
	DNJia FFPE Tissue Kit	DN983057	50 preps
	Hashin	LD983003	2ml
	RJ-Protease, Recombinant (20mg/ml)	EB983121	1ml

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; +982191070705.
- 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.

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 Blood and Cell 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 10^6	15-50µg	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

Sample preparation

First, resuspend the cell in flask, and 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.

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

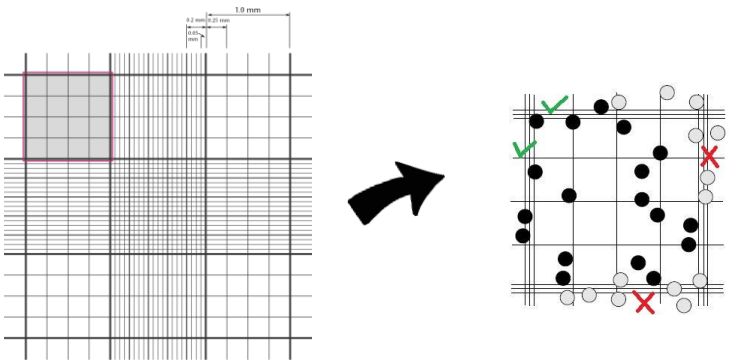

Figure1: Cell counting with hemocytometer

Factory address:

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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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