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**ROJETECHNOLOGIES**

# **MiRJia Lipid Kit**

RNA and total RNA isolation based on silica technology

- MiniPrep

## **For RNA Isolation from**

Animal Adipose Tissue

**By ROJE**  
**Edition, 01/2022**

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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## Kit Content

Component	100 preps
<b>RNSol H Reagent</b>	100ml
<b>TWB1 (concentrate)</b>	2 x 16ml
<b>TWB2 (concentrate)</b>	2 x 15ml
<b>Nuclease-free Water</b>	10ml
<b>HiPure DR Column</b>	100
<b>Collection Tube</b>	100

## Storage

Shipment condition is checked by ROJETechnologies. After arrival, RNSol H Reagent should be kept dry at 2-8 °C and all remaining reagent should be kept at room temperature (15-25 °C). When storage condition is as directed, all reagents are stable until expiration date, as indicated on the kit box.

## Intended Use

MiRJia Lipid Kit provides the components and procedures necessary for purifying RNA and miRNA from animal tissue and cell, PBMC, whole blood, WBC and bacteria cells. Notice that, MiRJia Lipid Kit is intended for molecular biology applications not for diagnostic use. We recommend all users to study RNA experiments guideline, before starting their work.

## Guarantee & Warranty

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 out 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](http://www.rojetechnologies.com)

## **Quality Control**

MiRJia Lipid 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.rojetechnoloos.com](http://www.rojetechnoloos.com)

## **Description**

MiRJia Lipid Kit provides a timesaving, reliable and meticulous method for RNA isolation from adipose animal tissues and cells. MiRJia Lipid Kit is based on HiPure DR Column technology for isolation of concentrated, highly purified and intact total RNA, which is suitable to be used for a variety of downstream applications such as northern blot experiments, real-time PCR, RNA sequencing, microarray etc.

## **Procedure**

The MiRJia Lipid Kit is designed for isolating all form of RNA molecules from 0.5-100mg tissue samples. Fresh or frozen tissue samples should be lysed and homogenized. Aqueous phase is separated using chloroform. Then, RNA binding to the silica membrane is achieved selectively, by the addition of ethanol to the lysate. Contaminants are removed by two specific kind of washing buffers. Pure RNA is finally eluted in nuclease-free water. Isolated RNA is ready to use in downstream applications. It has A260/A280 ratios of 1.9–2.3 by spectrophotometer, confirming high purity.

## **Equipment & Reagents to Be Supplied by User**

- Absolute ethanol
- 1.5ml RNase free microcentrifuge tubes
- RNase free pipets and pipet tips
- Benchtop refrigerated microcentrifuge (while RNSol H Reagent is used)
- Vortex
- Thermoblock or water bath

- TissueLyser/ Mortar and pestle (for animal tissue sample)
- Syringe and needles
- Homogenizer

## Applications

The isolated RNA and miRNA can be used in many downstream applications:

- RNA Sequencing
- cDNA synthesis
- Poly A+ RNA selection
- Microarrays
- RT-PCR and real-time RT-PCR
- Northern, dot blot analysis

## Features

Specific features of MiRJia Lipid Kit are listed here in Table 1.

**Table 1.** MiRJia Lipid Kit features and specifications

Features	Specifications
Elution volume (μl)	30-100μl
Technology	Silica technology
Main sample type	Animal adipose tissue
Processing	Manual
Purification of total RNA, miRNA, Poly A+ mi-RNA, DNA or protein	Yes
Sample Amount	0.5-100μg
Operation time per reaction (min)	Less than 45 min
Typical yield (μg)	2-20 μg
Average purity (A260/A280, A260/A230)	A260/A280= 1.9-2.3
Size of purified RNA	RNA ≥200 nt
Enzyme	No

## Recommended Starting Material

To reach optimized results it is better to follow as listed below. The size of the recommended starting material to use with determined RNSol H Reagent, chloroform and ethanol volumes are written in Table 2.

**Table 2.** Appropriate sample size and amount of RNSol H Reagent, chloroform amount for animal adipose tissue

Sample Size	RNSol Amount	Chloroform Amount
10 mg Animal Adipose tissue	100µl	100µl
20 mg Animal Adipose tissue	200µl	
30 mg Animal Adipose tissue	300µl	
40 mg Animal Adipose tissue	400µl	
50 mg Animal Adipose tissue	500µl	200µl
60 mg Animal Adipose tissue	600µl	
70 mg Animal Adipose tissue	700µl	
80 mg Animal Adipose tissue	800µl	
90 mg Animal Adipose tissue	900µl	
100 mg Animal Adipose tissue	1ml	

## Sample Storage and Preparation

### Animal Tissue

**Storage:** Fresh or frozen tissues may be used for the procedure. For long storage of tissue samples, Tissues should be flash-frozen in liquid nitrogen and transferred immediately to a -70 °C freezer. Tissues may be stored at -70 °C for several months. When isolating RNA and miRNA from frozen tissues ensure that the tissue does not thaw during weighing or prior to RNA preparation. It is recommended to store tissue sample in RNaseLag for storage (refer to RNaseLag).

**Preparation:** Sample preparation is composed of 2 steps, disruption and homogenization. First step is disruption of cell walls, plasma membranes of cells and organelles. Inefficient disruption results in RNA yield reduction. This can be done with one or cooperation of two methods depending on the tissue type. These methods include RNSol H Reagent, Mortar and



Pestle, Tissuelyser etc. Viscosity reduction can be accomplished by Homogenization. The aim is to create a homogeneous lysate. Sometimes these 2 steps happen simultaneously. For more information refer to Table 3.

**Note:** After disruption and homogenization in or RNSol H Reagent (lysis buffer), samples can be stored at  $-70^{\circ}\text{C}$  for months.

**Table 3.** Disruption and homogenization for different sample types

Sample type	Disruption	Homogenization
Animal Tissue	TissueRuptor/TissueLyser Mortar and pestle	TissueRuptor/TissueLyser Syringe and needles

## RNaseLag

For optimum result, it is recommended to store tissue samples in RNaseLag. RNaseLag is a reagent which stabilizes RNA in tissues and cells.

## Procedure

- Cut the animal tissue sample into slices less than 5mm thick, as quickly as possible.
  - Completely immerse the tissue pieces in the collection vessel containing RNaseLag.
- Note:** Make sure to use the appropriate volume of RNaseLag, so weight your sample before starting the procedure and use  $10\mu\text{l}$  RNaseLag per 1mg of tissue.
- The sample is ready for archival storage at conditions shown in Table 4.
  - After storage, for RNA isolation continue with appropriate protocol for the chosen sample type.

**Table 4.** Storage conditions and procedures after RNaseLag treatment.

Storage condition	Protocol
$2-8^{\circ}\text{C}$	Incubate the prepared sample (in RNaseLag) for up to 4 weeks at $2-8^{\circ}\text{C}$ .
$15-25^{\circ}\text{C}$	Incubate the prepared sample (in RNaseLag) for up to 7 days at $15-25^{\circ}\text{C}$ .
$37^{\circ}\text{C}$	Incubate the prepared sample (in RNaseLag) for up to 1 days at $37^{\circ}\text{C}$ .
$-20^{\circ}\text{C}$	First, incubate the prepared sample (in the RNaseLag) overnight at $2-8^{\circ}\text{C}$ . Then transfer it to $-20^{\circ}\text{C}$ for storage.
$-80^{\circ}\text{C}$	First, incubate the prepared sample (in the RNaseLag) at $2-8^{\circ}\text{C}$ . Then remove the tissue from the reagent, and transfer it to $-80^{\circ}\text{C}$ for long storage.

## Genomic DNA Contamination

MiRJia Lipid Kit is designed to selectively isolate RNA. However, if further DNA removal is intended, it is recommended to use DNase treatment kits, which are available from different suppliers.

## Before start

- Not forget to add the appropriate amount of ethanol (%96–100) to TWB1 and TWB2 as indicated on the bottle, before using for the first time.
- Do not forget to prepare %70 ethanol (nuclease-free).

## Maximize RNA Yield

To obtain higher yield of RNA, it is important to follow protocols carefully and pay attention to sample size table and its suitable lysis buffer amount recommended for your samples. Notice that all samples must be completely homogenized during cell lysis for maximum yields. It is good to know that:

- Yield and quality of the purified RNA depends on sample storage conditions. For best results, it is recommended to use fresh samples, however for long storage, it is better to treat sample in RNaseLag (for more information refer to RNaseLag).
- Avoid freezing and thawing samples, which may result in decreasing RNA yield, as compared to isolating RNA from fresh samples.

## 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 5. 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 5:** Washing buffer preparation

Buffer Name	Concentrated Volume	Amount of Ethanol	Final Volume
TWB1	16ml	24ml	40ml
TWB2	15ml	45ml	60ml

## Protocols Phenol-Chloroform Based Protocols

### Protocol: Isolation of Total RNA (Animal adipose tissues fresh and frozen)

**Sample Type:** Animal adipose tissues (fresh and frozen)

#### Some Tips to Know:

- All steps, before applying sample into HiPure DR Column, are carried out on ice.
- Not forget to add the appropriate amount of ethanol (%96–100) to TWB1 and TWB2 as indicated on the bottle, before using for the first time.
- For frozen samples, thaw them to room temperature. Avoid repeated thawing and freezing of samples due to decreasing in RNA yield.
- If working with RNA for the first time, read appendix 1 carefully.

#### Process

- Remove the tissue from RNaseLag or use fresh tissue. Determine the Weight of starting material. Add appropriate amount of RNSol H Reagent to the tissue sample (refer to the Table 2).
- Disrupt and homogenize the tissue sample by selecting one of these ways:
- After adding appropriate amount of RNSol H Reagent, use Micropestle followed by homogenizer or syringe needle to homogenize the tissue.
- After adding appropriate amount of RNSol H Reagent, using TissueLyser or homogenizer to disrupt and homogenize the sample simultaneously.
- Grind the weighed tissue in liquid nitrogen carefully with a cold mortar and pestle. Transfer tissue powder into an RNase-free, liquid-nitrogen-cooled, 1.5ml microcentrifuge tube. Let the liquid nitrogen to evaporate completely (but do not allow the tissue to thaw). Add the appropriate volume of RNSol H Reagent (refer to the Table 2) and homogenize by passing the lysate 5-10 times through a blunt 20-gauge needle fitted to an RNase-free syringe.

**Optional:** Homogenizing step is optional. It means that using homogenizer or syringe needle to homogenize the lysate can be omitted from the process.

- Incubate at room temperature for 5min.
- Add appropriate amount of chloroform (refer to Table 2), vigorously shake it for 30 s. Then pulse vortex for 15 s and incubate at room temperature for 3min.
- Centrifuge at 4°C for 15min at 13000 rpm.

- Transfer the aqueous phase (the upper phase) to a new tube. Be careful to avoid interfering the interphase.
- Add one volume of absolute ethanol to the separated aqueous phase. Pulse vortex for 30 s.
- Transfer the solution to a HiPure DR Column placed in a 2ml collection tube (supplied in the kit box). Centrifuge for 1min at 13000 rpm at room temperature. Discard the flow-through.
- Add 700µl TWB1 to the HiPure DR Column. Centrifuge for 1min at 13000 rpm at room temperature. Discard the flow-through.
- Add 500µl TWB2 to the HiPure DR Column. Centrifuge for 1min at 13000 rpm at room temperature. Discard the flow-through.
- Add 500µl TWB2 to the HiPure DR Column. Centrifuge for 3min at 14000 rpm at room temperature. Discard the collection tube with the flow-through.
- Place the HiPure DR Column in a new 1.5ml microtube. Add 30-100µl RNase-free water directly to the HiPure DR Column membrane. Centrifuge for 1min at 12000 rpm to elute the RNA.

**Note:** If the expected RNA yield is more than the yield from pervious step, put the HiPure DR Column on a new microtube and add another 30-100µl RNase-free water. Centrifuge for 1min at 12000 rpm. The yield will be nearly same as previous step. However, it is possible to pass the flow-through from step 12 once more to obtain RNA with higher concentration.

## 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
<b>Low yield</b>	Inappropriate sample storage condition	<ul style="list-style-type: none"> <li>Avoid freezing and thawing of samples, which results in reduced RNA Yield.</li> <li>For better results, it is recommended to store samples in RNaseLag.</li> </ul>
	Incomplete cell lysis	<ul style="list-style-type: none"> <li>Too much starting material results in low RNA yield. To optimize the results, refer to Table 2.</li> </ul>
	Ethanol from the washing buffer is present in elution	Preform another centrifugation before rehydration step to ensure no remaining trace of Ethanol on column. Carefully remove the column from the collection tube so that the column does not contact the flow-through.
	RNA elution is incomplete	<ul style="list-style-type: none"> <li>Perform rehydration step once more, by adding another 30-100µl rehydration buffer to the column and before centrifugation, incubate 5min at Room Temperature.</li> <li>Check that all previous steps are done appropriately.</li> </ul>
<b>Degradation</b>	Too thick sample for stabilization	<ul style="list-style-type: none"> <li>Cut large samples into slices less than 5 mm thick for stabilization in RNaseLag.</li> </ul>
	Improper sample storage	<ul style="list-style-type: none"> <li>It is suggested to store samples in RNaseLag, refer to sample preparation section.</li> </ul>
	Frozen sample used for stabilization	<ul style="list-style-type: none"> <li>For stabilization in RNaseLag, Use fresh samples.</li> </ul>
	Storage duration in RNaseLag exceeded	<ul style="list-style-type: none"> <li>Refer to Table 4.</li> </ul>

	RNase contamination	<ul style="list-style-type: none"> <li>All buffers have been tested and are guaranteed RNase-free. RNases can be introduced during use. Refer to appendix 1 for more information.</li> </ul>
<b>Low 260/280 ratio</b>	Insufficient disruption and homogenization	<ul style="list-style-type: none"> <li>As a guide for better disruption and homogenization, based on sample type refer to sample preparation guidelines.</li> </ul>
	RNA was diluted in low pH water	<ul style="list-style-type: none"> <li>Use 10 mM Tris-HCl with pH <math>\geq 7.5</math>, or nuclease free water with pH <math>\geq 7.5</math>.</li> </ul>
	DNA contamination	<ul style="list-style-type: none"> <li>Follow precisely the respective protocol, If RNA purification is still problematic further do DNase Treatment.</li> <li>RNSol pH might alter during storage. Check the RNSol H Reagent pH it should be around 4.2.</li> </ul>
	Protein contamination	<ul style="list-style-type: none"> <li>This is often due to exceeding the amount of starting material. Follow precisely the respective protocol, If RNA purification is still problematic further reduce the amount of starting material.</li> <li>Remove the aqueous phase precisely.</li> </ul>
<b>DNA contamination in downstream application</b>	No DNase treatment	<ul style="list-style-type: none"> <li>Perform DNase treatment.</li> </ul>
	No incubation with TWB1	<ul style="list-style-type: none"> <li>Incubate the spin column for 5min at room temperature after addition of TWB1 and before centrifuging</li> </ul>
	Check RNSol pH	<ul style="list-style-type: none"> <li>RNSol H Reagent pH might alter during storage. Check the RNSol pH it should be around 4.2.</li> </ul>
<b>Not performing well in downstream application</b>	Ethanol carryover	<ul style="list-style-type: none"> <li>Perform another centrifugation before rehydration step to ensure no remaining of Ethanol on column.</li> </ul>
	Salt carryover	<ul style="list-style-type: none"> <li>Between washing steps, eliminate remaining flow-through from the rim of collection tube, by blotting on clean paper towels.</li> </ul>
<b>Clogged Column</b>	Maximum amount of tissue exceeds kit specifications	<ul style="list-style-type: none"> <li>Refer to specifications to determine if the amount of starting material falls within kit specifications.</li> </ul>

	The sample is too large	<ul style="list-style-type: none"> <li>Use fewer 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</li> </ul>
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## Ordering Information

Category	Product Name	Cat No.	Size
	MiRJia Lipid Kit	RN003078	100 preps
	RNJia Kit	RN983006	100 preps
	RNJia Fibrous Kit	RN983025	50 preps
	RNJia Bacteria Kit	RN983022	50 preps
	RNJia Phenol-Free PB Kit	RN983057	100 preps
	RNJia Lipid Kit	RN983015	50 preps
	MiRJia Kit	RN003079	100 preps
	RNaseLag	RN983016	50ml
	RNZO	RN983048	25ml
	RNA Loading Set	LD983007	100 Preps
	RNSol H Reagent	RN983061	100ml

## 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](http://www.rojetechnologies.com)).
- Or send your questions to this email address, [technicalsupport@rojetechnologies.com](mailto:technicalsupport@rojetechnologies.com).

## Appendix 1: Handling RNA

RNA is highly sensitive to degradation due to the ubiquitous presence of RNases in the environment. Unlike DNases, which require metal ions for activity, RNases do not require cofactors for their enzymatic activity and can maintain activity even after prolonged boiling or autoclaving. Since RNases are active enzymes, and are too hard to be deactivated, do not use any plasticware or glassware without first removing possible RNase pollution. Special



precautions should be taken when working with RNA. All reagents and equipment must be specially treated to inactivate RNases prior to use.

The following are some tips and techniques to remember when working with RNA:

### **General Tips**

- Always wear latex or vinyl gloves while handling reagents and RNA samples to prevent RNase contamination from the surface of the skin or from dusty laboratory mobilizations. These materials are generally RNase-free and do not require pretreatment to inactivate RNase.
- Treat surfaces of benches with commercially available RNase inactivating agents such as RNZO (Order by cat No RN983018 and RN983019).
- Treat non-disposable glassware and plasticware before use, to ensure that it is RNase-free. Thoroughly rinse plasticware with 0.1N NaOH/1mM EDTA, and RNase-free water.
- Use disposable RNase-free plasticware to decrease the possibility of sample contamination.
- Keep purified RNA on ice when aliquots are pipetted for downstream applications.
- Glassware should be immersed in freshly prepared %0.1 (v/v) DEPC in water or ethanol for several hours, followed by draining and autoclaving.

### **Appendix 2: RNA Storage Condition**

RNA samples are commonly stored at  $-20^{\circ}\text{C}$  or  $-80^{\circ}\text{C}$ , eluted in RNase-free water. Under these conditions, RNA is not degraded after 1 year.

#### **RNA Quality**

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

### **Appendix 3: RNA Integrity**

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

twice the intensity of the lower band (18S in eukaryotic cells and 16S in bacterial cells) and should be sharp and tight.

### Denaturing agarose gel electrophoresis

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

### Gel preparation

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

### RNA sample preparation

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

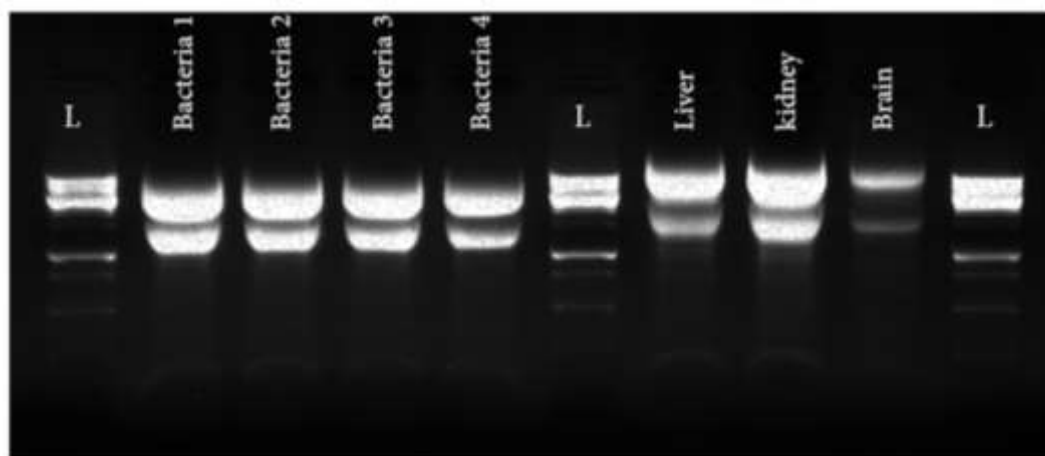
### Electrophoresis

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

### Results

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

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



**Figure 1.** Isolated RNA using RNJia Kit, RNA was isolated from the indicated animal tissues and bacteria cells using the RNJia Kit. For each sample, 10 µg RNA was pretreated by ROJE Sample Buffer and analyzed on Denaturing Gel Electrophoresis (DGE).

## Appendix 4: DNA Contamination

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

strategies to solve the problem:

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

## Appendix 5: Convert RPM to RCF (centrifuge)

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

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

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

## Appendix 6: Cell Count by a Hemocytometer

### 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 0.5ml 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.

### **Loading Sample**

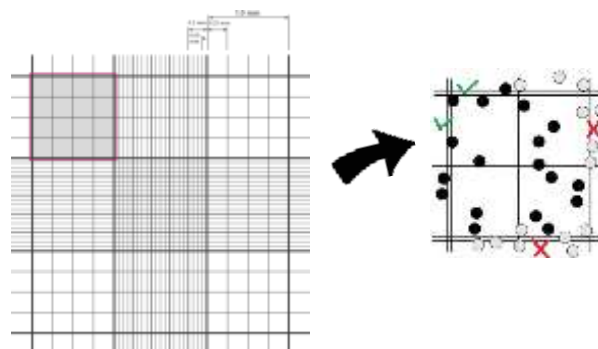
Load your sample quickly and smoothly. It is important to handle the hemocytometer and the cover slip carefully. Never place your fingers on the reflective surface of the slide. Always clean the slide before you load the sample by rinsing the slide and cover slip with %70-95 ethanol. Air-dry or gently 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  $\mu$ l 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 2). 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  $\times$  dilution factor  $\times 10^4$



**Figure2.** Cell counting with hemocytometer

## Appendix 7: Preparation of Phosphate Buffered Saline (PBS)

Dissolve the components in about 800 mL dH<sub>2</sub>O. Adjust the pH to 7.4 with HCl. Adjust the volume to one liter. Sterilize by autoclaving. Store at room temperature.

**Table 6.** PBS preparation

Concentration	Component	g/L
137mM	NaCl	8g
2.7mM	KCl	0.2g
10mM	Na <sub>2</sub> HPO <sub>4</sub>	1.42g
1.8mM	KH <sub>2</sub> PO <sub>4</sub>	0.25g

## Factory address

NO. 2 Farvardin street- Fernan Street- Tehran- Shahr Qods- Iran- Postal Code: 37531146130-  
phone: +982191070705

**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.

Factory address:

No. 2, Farvardin St., Fernan St.,  
Shahr-e-Qods, 3753146130, Tehran, IRAN.

Tel: 021 91070705