Medical Laboratory Techniques Department

Title :- RNA isolation and Quantification of DNA & RNA concentration with Nanodrop

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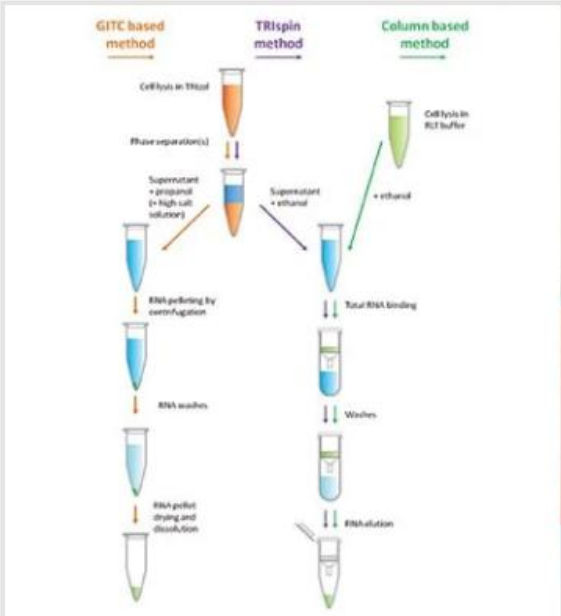
Lab 4

Principle of RNA Isolation

Total **RNA** is isolated and separated from DNA and protein after extraction with a solution called as Trizol. Trizol is an acidic solution containing guanidinium thiocyanate (GITC), phenol and chloroform. GITC irreversibly denatures proteins and RNases. This is followed by centrifugation. Under acidic conditions, total RNA remains in the upper aqueous phase, while most of DNA and proteins remain either in the interphase or in the lower organic phase. Total RNA is then recovered by precipitation with isopropanol. RNase enzymes can be inactivated by including diethyl pyrrocarbonate (DEPC).



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Materials Required for RNA Isolation

1- Bacterial culture
2- Trizol
3- Chloroform
4- Isopropanol solution
5- TAE buffer
6- 70% ethanol

Procedure of RNA Isolation

1- Take 800 µL of bacterial culture in a fresh eppendorf.

2- To this add 160 µL of Trizol (1/5th of culture volume).

3- The solution was mixed well by pipetting several times.

4- To this add 32 µl of chloroform (1/5th volume of trizol).

5- Incubate for 2 to 5 minutes and centrifuge at 12000 rpm for 15 minutes at 4° C

6- Transfer the aqueous phase into a new tube and add equal volume of isopropanol. Mix well.

7- Centrifuge at 10000 rpm for 10 minutes at 4° C.

8- Discard the supernatant and resuspend the pellet in 70% ethanol. • Again centrifuge at 10000 rpm for 10 minutes at 4° C.

9- Discard the supernatant.

10- Air dry the pellet at 37° C for 10-15 minutes.

11- Resuspend the pellet in 50 µL of TE buffer.

12- Analyse the RNA sample quantitatively and qualitatively

Quantitative estimation

Quantitative estimation of (DNA) using spectrophotometer:

DNA concentration can be measured using spectrophotometer (SmartspecTM 3000, Bio-Rad). DNA in 100 µl sample is quantified using spectrophotometer. Two microliters (µl) of DNA is diluted in 98 µl water and absorbance is measured at wavelength 260 nm and 280 nm. Calculation of the approximate quantity of the nucleic acid in the sample is determined using the following formula:

Amount of DNA in ng/µl =Absorbance at 260 of sample × 50 (Maniatis et al., 1989). Similarly, it gives the A260/280. Any sample whose A260/280 ratio is < 1.7 or >1.9 is not further processed.

Quantitative estimation of (RNA) using spectrophotometer

The RNA concentration is determined by measuring absorbance at 260 nm on a spectrophotometer (one absorbance unit = 40 µg/ml RNA). The A260/A280 ratio should be approximately 2.0, but figures between 1.8 and 2.1 are considered acceptable. After measuring the optical densities the concentration of RNA can be calculated as follows: [RNA] (µg/ml) = 40 x Dilution Factor x OD260.

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Quantification of nucleic acid (DNA/RNA) using NanoDrop

Nucleic acids absorb light at a wavelength of 260 nm. If a 260 nm light source shines on a sample, the amount of light that passes through the sample can be measured, and light absorbed by the sample can be inferred. For double stranded DNA, an Optical Density (OD) of 1 at 260 nm correlates to a DNA concentration of 50 ng/µl, so DNA concentration can be calculated from OD measurements.

Nano Drop

Nucleic Acid Calculations:

For nucleic acid quantification, the Beer-Lambert equation is modified to use a factor with units of ng-cm/microliter. The modified equation used for nucleic acid calculations is the following:

$c=(A\times \varepsilon/b$

c = the nucleic acid concentration in ng/microliter

A = the absorbance in AU

ε = the wavelength-dependent extinction coefficient in ng-cm/microliter

b= the pathlength in cm.The generally accepted extinction coefficients for nucleic acids are: Double-stranded DNA: 50 ng-cm/Ml

Procedure:

Step1: **Initialization of the NanoDrop:**

1- Open and turn on the computer attached to the NanoDrop and lift the upper arm of the NanoDrop and remove the Lab wipe from the pedestal.

2- Add 4-5 µl of purified water to the lower pedestal, and then lower the arm, Wait for 30-60 seconds. Lift the upper arm and wipe both the upper and lower pedestals.

3- Open the NanoDrop software on the computer

4- Initialize the NanoDrop.

5- Click on the "Nucleic Acid" button in the Nano Drop software.

Step 2 Blanking the Nano Drop

1- Add 2 µl of the buffer or autoclaved water depending on the sample in use (if DNA pellet suspension in TE/Water)

2- Lower the upper arm of the NanoDrop and click the "Blank" button on the software.

3- Wait for 20 seconds for the blank measurement to be made. When it is done, lift the upper arm and dry the pedestal with a wipe.

Step-3 Sample Measurement

1. Add 2 µl of the sample to the lower pedestal, and then lower the upper arm.
2. In the "Sample ID" box, type in the name of the sample.
3. Clicked the "Measure" button on the software and wait for 20 seconds for measurement. When it's done, lift the upper arm and dry the pedestal.
4. Repeat the above steps 3- substeps to measure all the samples.
5. Save the data file for records and close the nanodrop software.

Step-4 Cleaning the pedestal:

1. Add 4-5 µl of purified water to the lower pedestal, then lower the arm. Wait for 30-60 seconds.
2. Lift the upper arm and use a wipe to vigorously scrub both the upper and lower pedestals.
3. Place a new folded lab wipe on the lower pedestal and closed the upper arm until used next time.