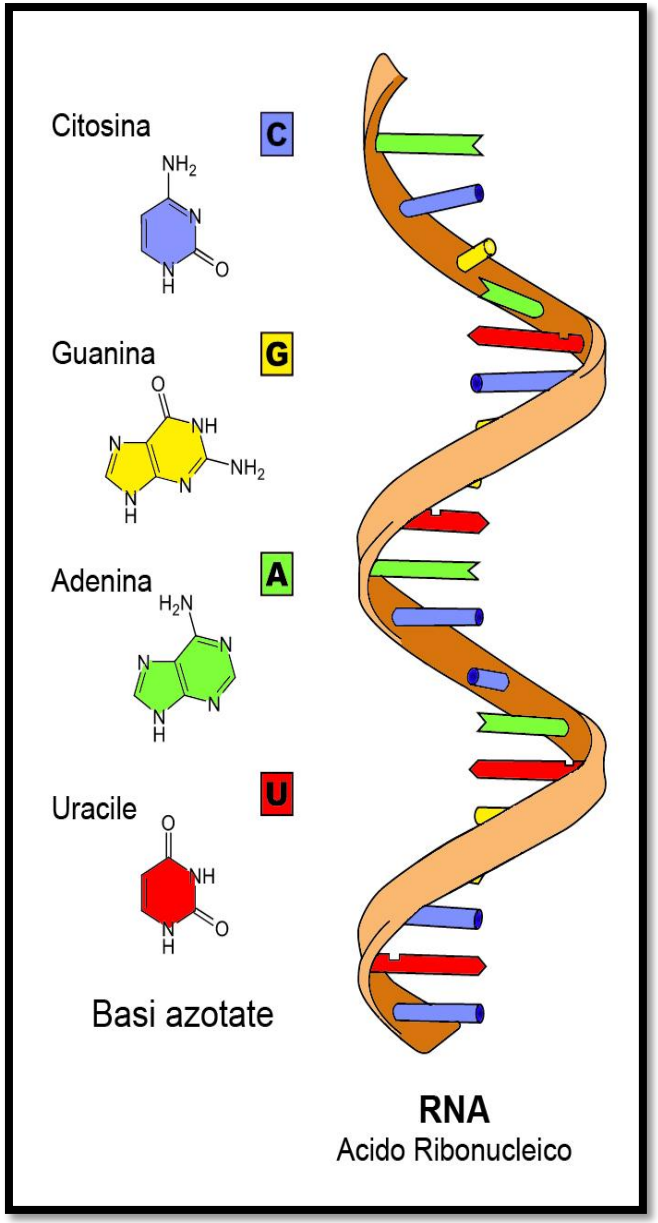
Ribonucleic acid (RNA)

- **Ribonucleic acid** (**RNA**) is more often found in nature as a single-strand folded onto itself. Cellular organisms use messenger RNA (mRNA) to convey genetic information (using the nitrogenous bases guanine, uracil, adenine, and cytosine) that directs synthesis of specific proteins.
- Many viruses encode their genetic information using an RNA genome.



Types of RNA
1. Messenger RNA (mRNA)

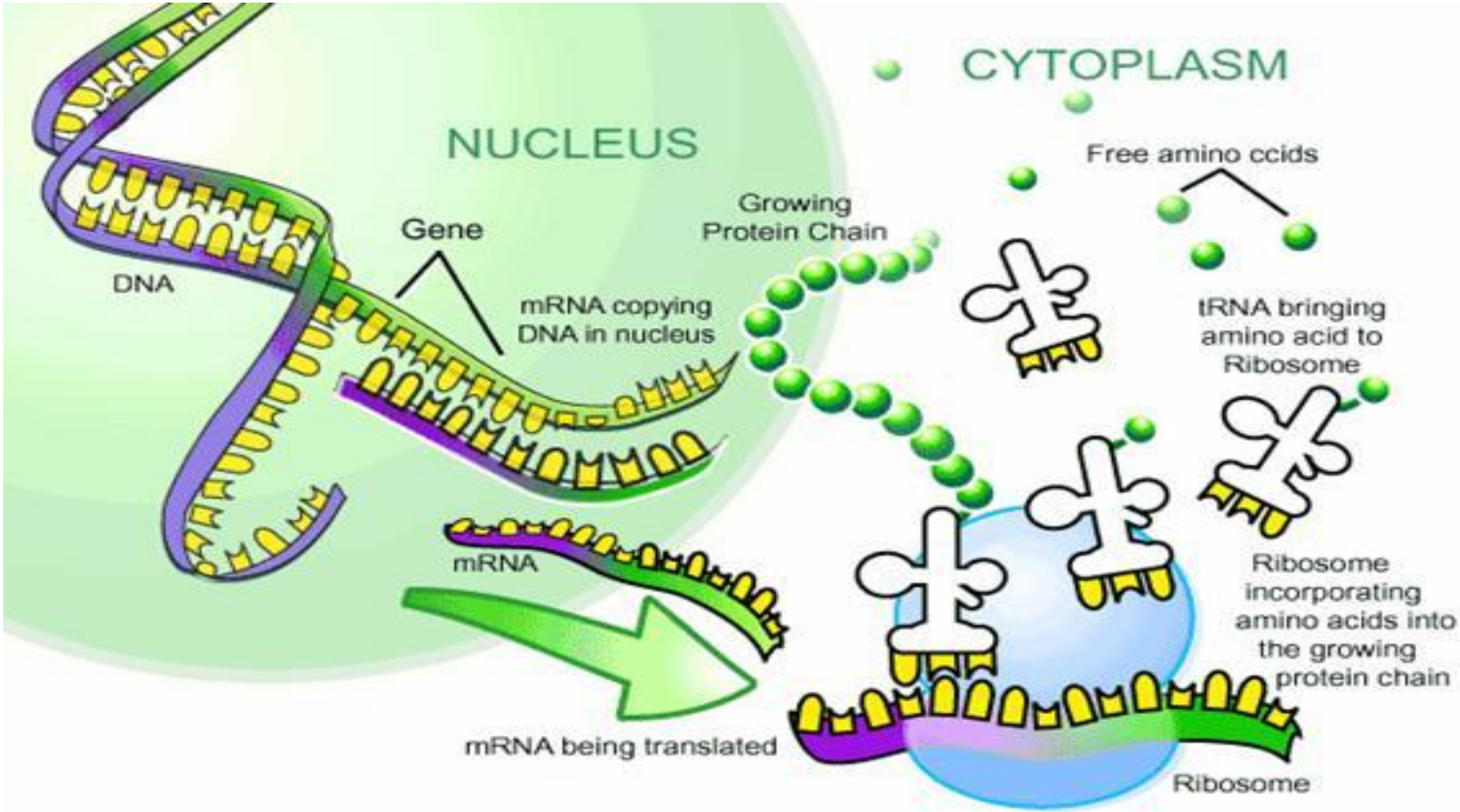
- **Messenger RNA** (**mRNA**) is a large family of RNA molecules that convey genetic information from DNA to the ribosome, where they specify the amino acid sequence of the protein products of gene expression. Following transcription of primary transcript mRNA by RNA polymerase, processed, mature mRNA is translated into a polymer of amino acids: a protein

2. Transfer RNA (abbreviated **tRNA**

- A **transfer RNA** (**tRNA**) is an adaptor molecule composed of RNA, typically 76 to 90 nucleotides in length, that serves as the physical link between the mRNA and the amino acid sequence of proteins.
- It does this by carrying an amino acid to the protein synthetic machinery of a cell (ribosome) as directed by nucleotide sequence (codon) in a messenger RNA (mRNA). As such, tRNAs are a necessary component of translation, the biological synthesis of new proteins according to the genetic code.

3. Ribosomal ribonucleic acid (rRNA)

- In molecular biology, **ribosomal ribonucleic acid** (**rRNA**) is the RNA component of the ribosome, and is essential for protein synthesis in all living organisms. It constitutes the predominant material within the ribosome.
- The ribosomal RNAs form two subunits, the large subunit (LSU) and small subunit (SSU). The LSU rRNA acts as a ribozyme


CYTOPLASM
NUCLEUS
Free amino ccids
Growing
Protein Chain
Gene
DNA
mRNA copying
DNA in nucleus
tRNA bringing
amino acid to
Ribosome
mRNA
Ribosome
incorporating
amino acids into
the growing
protein chain
mRNA being translated
Ribosome

RNA Extraction Protocol

Tissue

1. Upon extraction from the animal, immediately slice the tissue into pieces no wider than **0.5cm** and drop into RNA Later. (The volume of RNA Later should be at least ten times the volume of tissue)

2. Store the tissue (until homogenization) according to the following: Initially - overnight at 2-8 C, Then –indefinitely < -20 C, up to four weeks at 2-8 C, up to 7 days at 2-8 C, up to 1 day at 37 C.

3. Snap Freezing in Liquid Nitrogen:- Following immersion, keep the tissue in the Nitrogen until the procedure is completed.

4. Upon completion of the harvest procedure, transfer the tissues to empty falcon tubes stored on dry ice.

5. Keep the tissue frozen until the homogenization procedure is ready to be performed extraction of RNA Later:

PRINCIPLE

- The isolation of RNA with high quality is a crucial step required to perform various molecular biology experiment.
- TRIzol Reagent is a ready-to-use reagent used for RNA isolation from cells and tissues.
- TRIzol works by maintaining RNA integrity during tissue homogenization, while at the same time disrupting and breaking down cells and cell components.
- Addition of chloroform, after the centrifugation, separates the solution into aqueous and organic phases.
- RNA remains only in the aqueous phase.
- After transferring the aqueous phase, RNA can be recovered by precipitation with isopropyl alcohol. But the DNA and proteins can recover by sequential separation after the removal of aqueous phase.
- Precipitation with ethanol requires DNA from the interphase, and an additional precipitation with isopropyl alcohol requires proteins from the organic phase. Total RNA extracted by TRIzol Reagent is free from the contamination of protein and DNA.
- This RNA can be used in Northern blot analysis, in vitro translation, poly (A) selection, RNase protection assay, and molecular cloning

Materials Required

- **Reagents**

- Chloroform (without any additives, such as isoamyl alcohol)
- Isopropyl alcohol
- 75% Ethanol (in DEPC-treated water)
- RNase-free water or 0.5% SDS solution

Preparing Samples
Homogenizing samples

1. Determine your sample type, and perform homogenization at room temperature. The sample volume should not exceed 10% of the volume of TRIzol® Reagent used for homogenization. Be sure to use the indicated amount of TRIzol® Reagent, because an insufficient volume can result in DNA contamination of isolated RNA.

2. When preparing samples with high content of fat, proteins, extracellular material (e.g., muscle, fat tissue, or tuberous plant material), an additional isolation step may be required to remove insoluble material from the samples.

Note: **Do not** perform this additional isolation step if you are performing subsequent DNA isolation on your sample.

3. Proceed to **Phase separation**, or store the homogenized sample. Homogenized samples can be stored at room temperature for several hours, or at –60 to –70°C for at least one month.

Homogenization

1) For tissues that are snap frozen or slightly in excess, the homogenization of the tissue should be done by mortar and pestle (cooled to temp in a liquid nitrogen bath).

2) At the same time, transfer at least 1mL TRIZOL / 100mg tissue to be homogenized into a falcon tube

3) Transfer the tissue to the pestle and grind until a layer of very fine dust is all that is left.

4) Use an RNase free spatula to transfer the dust to the TRIZOL solution. Be sure to get as much dust as possible.

5) Vortex mixture thoroughly.

*For tissues that are very small or highly precious, a hand-held tissue grinder is recommended. The homogenization is performed in the presence of the 1mL TRIZOL / 100mg tissue until the tissue is completely dissolved in solution.

*For cultures of cells (suspended in solution), quantify, pellet the cells, and resuspend in TRIZOL at a volume of 5 x 10^6 cells / 1mL TRIZOL.

6) Once homogenized, aliquot the solution to eppendorf tubes and leave in TRIZOL at room temp for five minutes.


RNA Extraction by TRIzol ®
Abnova
TRIzol® Reagent
Add 1ml TRIzol® to the sample and homogenize
0:13 / 3:04

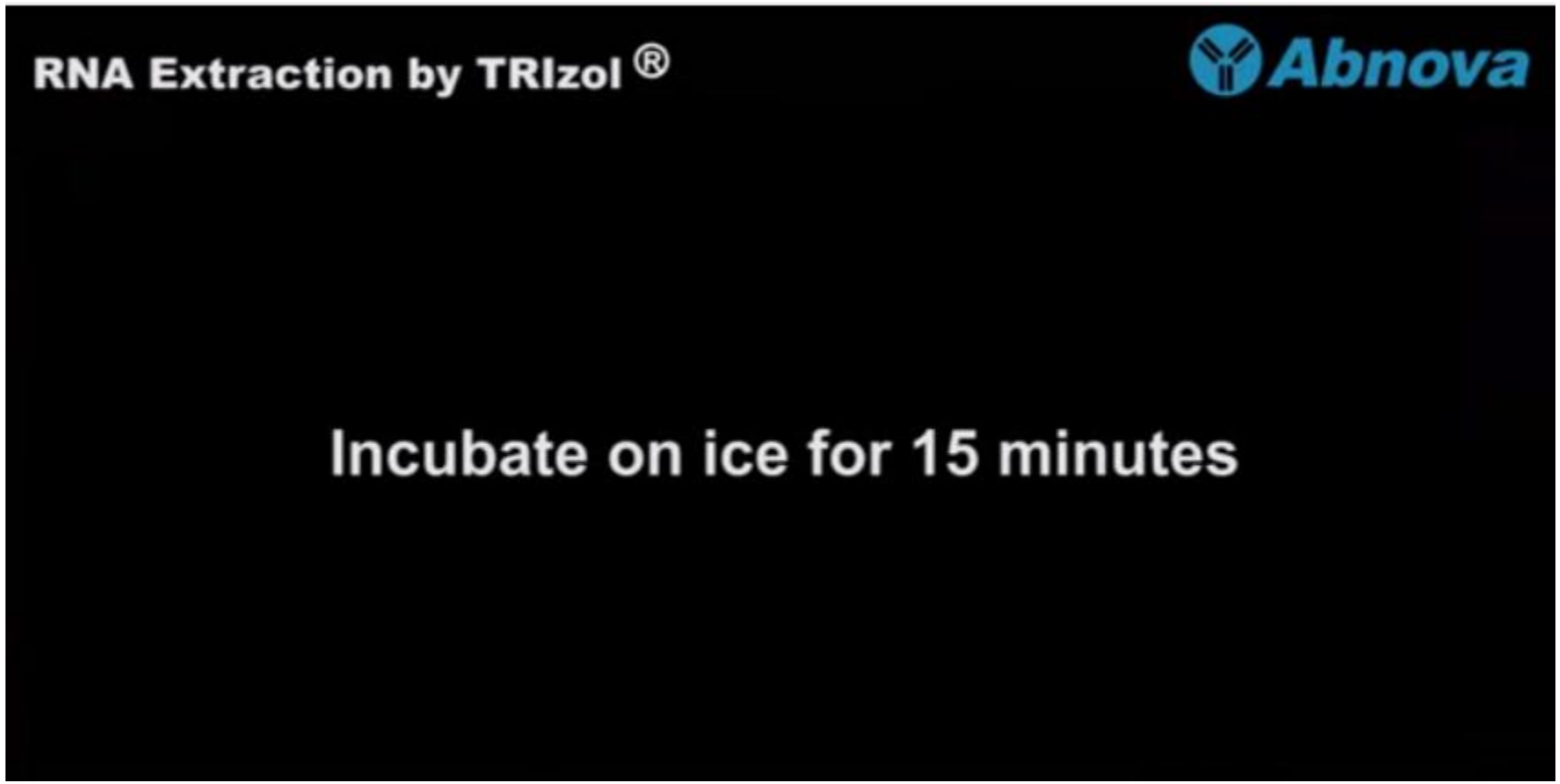

RNA Extraction by TRIzol ®
Abnova
Add 1ml TRIzol® to the sample and homogenize
0:19 / 3:04

2.PHASE SEPARATION

- The homogenized samples were incubated for 5 minutes at 15 to 30°C for the complete dissociation of nucleoprotein complexes.
- 0.2 ml (200 microliters)of chloroform per 0.75 ml of TRIZOL LS Reagent was added. The tubes were shaked vigorously by hand for 15 seconds and incubated them at 15 to 30°C for 2 minutes.
- The samples were centrifuged for 15 minutes at no more than 12,000 g (4°C).
- The aqueous phase was transferred to other tubes. (Following centrifugation, the mixture separates into a lower red, phenol-chloroform phase, an interphase, and a colorless upper aqueous phase. RNA remains only in the aqueous phase. The volume of the aqueous phase is about 70% of the volume of TRIZOL LS Reagent used for homogenization.)


RNA Extraction by TRIzol ®
Abnova
chloroform
Add 200µl chloroform to the homogenate
0:28 / 3:04


RNA Extraction by TRIzol ®
Abnova
Vortex vigorously
0:37 / 3:04


RNA Extraction by TRIzol ®
Abnova
Incubate on ice for 15 minutes

RNA Extraction by TRIzol ®

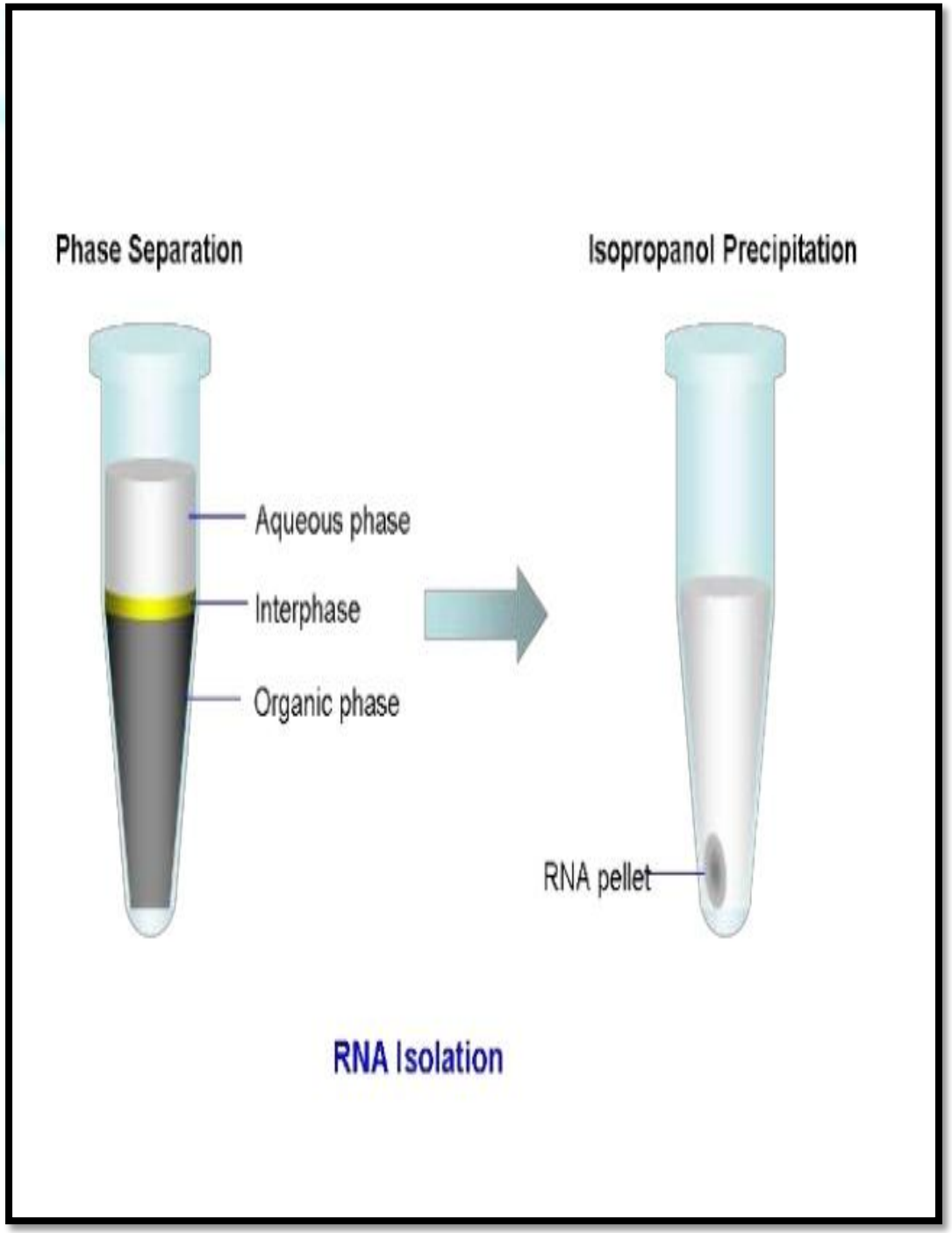
Abnova

Centrifuge to get phase separation (12,000g for 15 minutes at 4°C)


RNA Extraction by TRIzol ®
Abnova
Transfer the aqueous phase to a fresh tube

3. RNA Precipitation

- The RNA was precipitated from the aqueous phase by mixing with 3 microlitre of glycogen and 500 microlitre of isopropyl alcohol.
- The mixture was centrifuged for 30 minutes at 12,000 × g (2 to 8°C).(The RNA precipitate forms a gel-like pellet on the side of the tube at bottom).




RNA Extraction by TRIzol ®
Abnova
Isopropanol
Precipitate the RNA by mixing with 0.5ml isopropanol
Incubate on ice for 10 minutes


RNA Extraction by TRIzol ®
Abnova
Centrifuge for 10 minutes at 12000g at 4°C


RNA Extraction by TRIzol ®
Abnova
Remove the supernatant

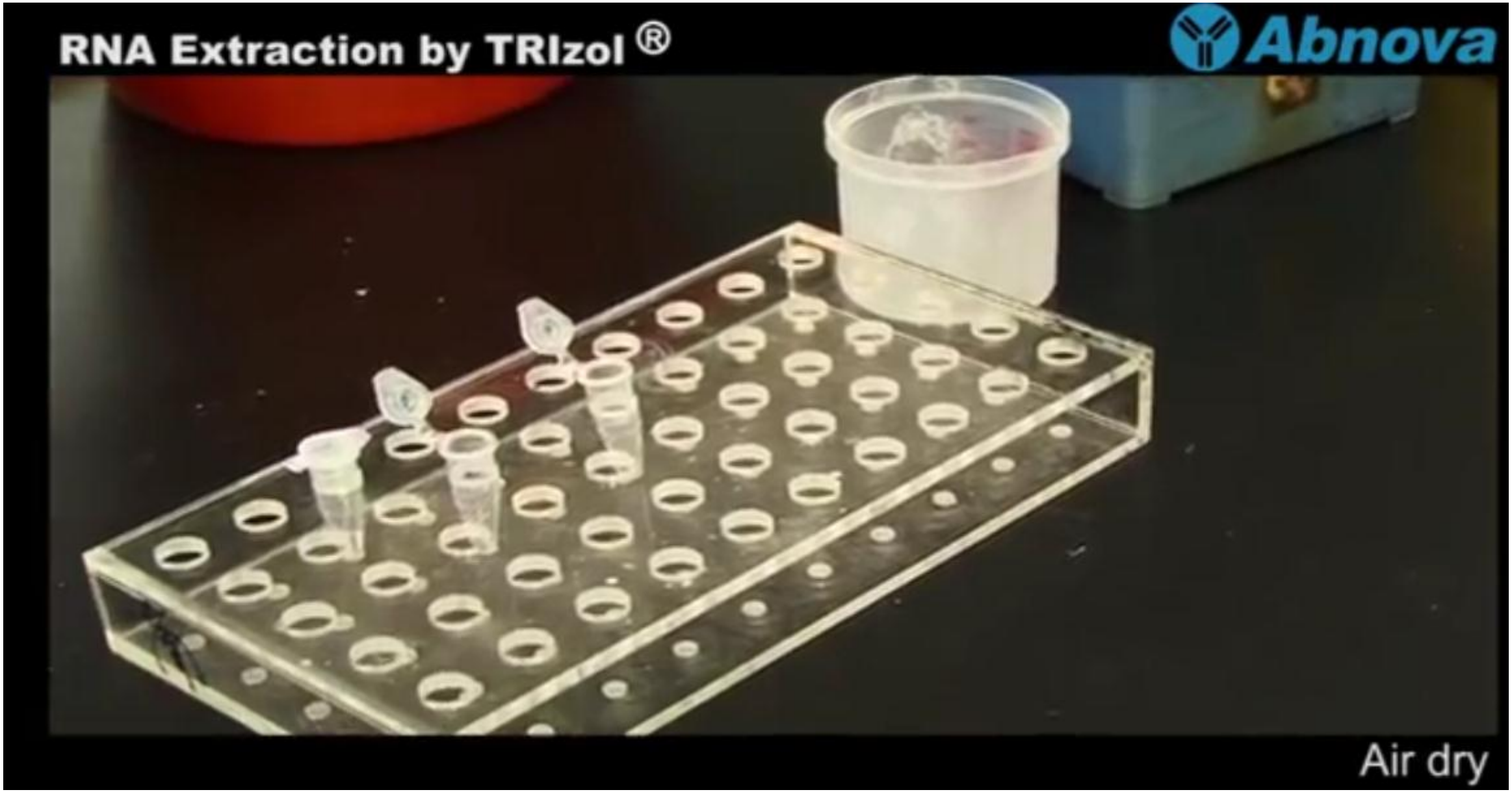
4. RNA Wash

- The supernatant was removed. The RNA pellet was washed once with 75% ethanol, adding 900 microlitre of 75% ethanol per 0.75 ml of TRIZOL LS Reagent used for the initial homogenization.
- The sample were inverted and mixed and centrifuged at 12,000 rpm for 30 minutes at 4 degree.


RNA Extraction by TRIzol ®
Abnova
70% EtOH
Wash pellet with 1 ml 70% ethanol by flicking


RNA Extraction by TRIzol ®
Abnova
Centrifuge at 7500g for 10 minutes at 4°C


RNA Extraction by TRIzol ®
Abnova
Remove the supernatant


RNA Extraction by TRIzol ®
Abnova
Air dry

5. Redissolving RNA

- The RNA pellet was dried .
- RNA was dissolved in RNase-free water (or 0.5% SDS solution) by passing the solution through the pipette tip for a few times, and incubating for 10 minutes at 55 to 60°C.


RNA Extraction by TRIzol ®
Abnova
Dissolve RNA pellet in appropriate volume of RNase-free H2O