

DNA-RNA Lab Kit Instruction Manual

General Information

Description

DNA-RNA Lab Kit is designed to enable your students learn real-world molecular biology techniques in a regular high school or college science classroom. The key component of the kit is AquaRNALite™, a nontoxic DNA-RNA extraction solution. This aqueous solution is the first ever reagent developed for RNA extraction without the use of toxic chemicals, such as phenol, chloroform, or guanidine isothiocyanate. In combination with AquaStain™, a nontoxic color DNA and RNA stain, your students will be able to extract DNA and RNA, separate the DNA and RNA by gel electrophoresis, and visualize the DNA and RNA under ambient lights without using the hazardous ethidium bromide and UV lights.

Specification

Product Name	DNA-RNA Lab Kit
Product #	7000
Kit Contents	Instruction Manual 30 ml AquaRNALite™ Solution (Product # 7030) 4x 1 ml AquaStain™ Solution (Product # 7115) 4x 0.5 ml AquaLoading™ Solution (Product # 7215)
MSDS	Available at www.aquaplasmid.com
Storage	Store tightly capped at RT (~22°C). Vortex to mix well before using.

Terms & Conditions

Product Usage: For In Vitro Laboratory Research and Education Use Only. NOT to be administered to humans or used for medical diagnosis.

Product Warning: May be harmful if swallowed. May cause irritation. Avoid contact with eyes and skin. Always wear gloves and safety goggles when handling any chemicals.

Limited Product Warranty: We offer a LIMITED PRODUCT WARRANTY to our customers. This warranty limits our liability to replacement of this product. No other warranties of any kind, express or implied, including without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by MultiTarget Pharmaceuticals. We shall have no liability for any direct, indirect, consequential, or incidental damages arising out of the use, the results of use, or the inability to use this product.

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Introduction

Molecular biology techniques are employed in every discipline and every area of life science. Learning these techniques would help your students understand many concepts taught in a high school and college biology curriculum and help prepare them for future majors and careers in biotechnology, including practice, research, and development in medical, pharmaceutical, veterinary, agricultural, and environmental sciences.

The core of modern molecular biology techniques lays on the isolation, analysis, and manipulation of DNA, RNA, and protein molecules. Until now, these techniques can only be learned and practiced in standard research laboratories, due to their requirement and use of toxic chemicals. We have developed a nontoxic, multifunctional, aqueous solution based reagent AquaRNALite™ for DNA and RNA extraction. None of the ingredients in AquaRNALite™ is classified as health or physical hazard or regulated. It is suitable for use in high school and college lab classrooms to give your students hands-on experience with basic molecular biology techniques. The extraction protocol is simple, easy, and fast. The cells are lysed with AquaRNALite™, the released DNA and RNA molecules are precipitated by isopropanol.

This manual describes the extraction of DNA and RNA from the harmless laboratory *E. coli* bacteria (strain K-12). However, the kit can also be used to extraction DNA and RNA from other biological samples, including cultured mammalian and plant cells, fresh animal tissues, fruits and vegetables from local grocery stores, or weeds and bugs (maybe politically incorrect, but should be allowed if your students have designed an inquiry-based project) in the backyards. For protocols, please refer to AquaRNA™ protocols on our website www.aquaplasmid.com.

In addition to AquaRNALite™, we have developed a nontoxic color DNA and RNA staining solution AquaStain™. DNA and RNA bands are stained during electrophoresis with AquaStain™ and visualized under ambient lights, avoiding the use of hazardous ethidium bromide and UV lights.

These lab activities will reinforce the topics you have taught in the classroom, such as scientific inquiry and research – designing different experiments by changing the subject, treatment, and observation; cell biology - eukaryotic and prokaryotic cell structure and organization; molecular biology – DNA, RNA, and protein (enzyme) structure and function; genetics - genes, chromosomes, genomes, and genetic inheritance and diseases.

This DNA-RNA Lab Kit will benefit your students – getting hands-on DNA/RNA lab experience; your school – offering advanced science curriculum; and you – aiding your professional development.

Lab 1. DNA and RNA Extraction From *Escherichia coli* Bacteria

E. coli bacterium is the working horse of molecular biology and biotechnology. Walking into any molecular biology lab, you will find scientists are working with *E. coli*. Because they can be multiplied to billions and trillions of the same exact cells overnight (fascinating isn't it!), *E. coli* bacteria are widely used to produce engineered DNA, RNA, and proteins by scientists. It is necessary for your students to learn to work with *E. coli* early on. The *E. coli* strains (K-12 and derivatives) used in research laboratories are non-pathogenic and harmless (Drink it if you like! Of course, not to recommended). They were originated from the normal flora in our intestines, but they have been modified and are unable to survive in the intestines or form biofilm in the environment, so they are safe to humans, animals, and the environment.

Required equipment and reagents

Included in the kit:

AquaRNALite™ - 30 ml (for 300 extractions)

Not included with the kit:

E. coli K-12 stain (1 stock, purchased from scientific supply stores)
LB broth capsules (250 g, purchased from scientific supply stores)
Glass bottles (250 and 500-ml, for preparing liquid LB broth and growing the bacteria)
Magnetic stirrer and stir bar (1 each, you may already have them in your chemistry lab)
Lysozyme (5 g, purchased from scientific supply stores)
TE buffer (100 ml, homemade or purchased from scientific supply stores)
1.5-ml clear microfuge tube (1 for each student)
20, 200, and 1000 µl adjustable Pipettors (1 set for each group of 6 students, ~\$100 each)
20, 200, and 1000 µl pipette tips (1 box each with 980 tips in each box)
2000-3000 rpm minicentrifuge (1 for each group of 6 students, ~\$200 each)
Mini vortex mixer (1 for each group of 6 students, ~\$200 each)
90-100% isopropanol (500 ml, purchased from a pharmacy)
50% isopropanol (500 ml, prepared in a squirt plastic bottle)
Deionized water (4x 500 ml, requested at your local research labs)

Ideas for lab curriculum extensions

- Try growing the *E. coli* bacteria in soda and sports drinks (you'll be amazed!)
- Discuss the difference of K-12 strain and the O157:H7 strain that causes food recalls
- Try extract *E. coli* DNA/RNA without breaking down the cell walls with lysozyme
- Extract DNA and RNA from other living things
- Strike and grow the bacteria on agar plates with and without antibiotics
- Perform a plasmid transformation experiment
- Do a PCR or RT-PCR reaction with the isolated DNA and RNA

Protocol For Extracting DNA and RNA from *E. coli* Bacteria

RNases are everywhere living things have touched. They would chew up your isolated RNA if they could get to it. Don't let your fingers touch the inside of the bottles, tubes, caps, and tips that may come in contact with your isolated RNA!

1. Harvest the Cells (Step 1 here was performed by your teacher the day before)

- 1) Inoculate 50 ml of LB broth in a 250-ml bottle with 0.5 ml of *E. coli* K-12 stock. Put in a 50% isopropanol sterilized stir bar and stir at room temperature with a magnetic stirrer continuously for 12-18 hours to grow the bacteria.
- 2) Transfer 1 ml of the bacterial culture into a 1.5-ml microfuge tube (Note: The remaining culture can be aliquoted into 1.5-ml microfuge tubes and stored in a freezer as bacteria stock for future uses. Use 10% bleach to rinse all the bottles and tips that have come in contact with the bacterial culture before cleaning or discarding them.)
- 3) Centrifuge for 4 minutes to pellet the bacteria. Flip the tube to discard the medium as completely as possible.

2. Extract the DNA and RNA

- 1) Add 100 µl of 1 mg/ml lysozyme in TE buffer pH 8 (the lysozyme solution was prepared earlier by your teacher, aliquoted, and stored in a freezer before use) to the bacteria pellet. Pipette and vortex vigorously to fully suspend the bacteria. Incubate at room temperature for 15 minutes to allow the enzyme to break down the bacterial cell walls.
- 2) Add 100 µl of AquaRNALite™ to the bacteria suspension. Vortex vigorously to mix well. Incubate at room temperature for 4 minutes to lyse the cells.

3. Pellet the DNA and RNA

- 1) Add 200 µl of 90-100% isopropanol to the lysate and mix the contents by vortexing for 30 seconds. You will now see the DNA and RNA (threads and aggregates) precipitating out of the solution.
- 2) Centrifuge for 4 minutes to pellet the DNA and RNA. Decant the tube to discard the supernatant. Make sure that you see the DNA/RNA pellet before and after discarding the supernatant.
- 3) Fill the tube with 50% isopropanol by shooting the isopropanol solution from a squirt bottle at the cap of the tube, and then flip the tube to discard the solution. Repeat the rinse once. Tap the tube upside down on a piece of clean paper towel to remove residual isopropanol and let it air dry for 4 minutes.
- 4) Add 100 µl of deionized water to the DNA/RNA pellet. Pipette and vortex vigorously for 2 minutes to fully suspend the DNA and RNA.
- 5) Store your DNA/RNA solution in a freezer (-20° C) until gel electrophoresis.

Lab 2. Agarose Gel Electrophoresis

Gel electrophoresis is one of the most commonly used techniques in molecular biology. Knowing the principle of gel electrophoresis will pay long-term dividend for your students to understand the mechanisms of many modern biotech analytical equipment and techniques, including sequencer, bioanalyzer, chromatography, Western, Northern, and Southern blots, DNA fingerprinting, and more. Agarose gel electrophoresis unit should be a must-have piece of equipment for every high school science lab. This lab activity allows your students to run the DNA and RNA they have isolated in an agarose gel electrophoresis and see the DNA and RNA bands right in front of their eyes (Figure 1).

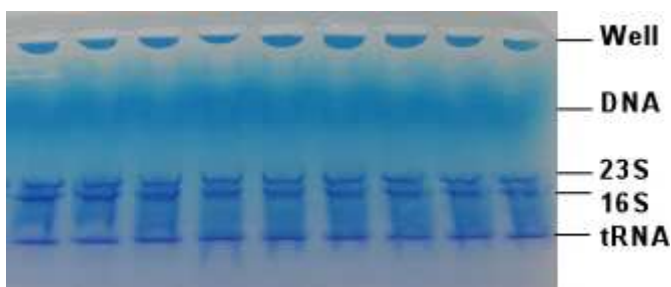


Figure 1. Agarose gel electrophoresis of DNA and RNA extracted from *E. coli* bacteria by AquaRNALite™.

Required equipment and reagents

Included in the kit:

AquaStain™ - 4x 1 ml, for DNA/RNA stain and visualization

AquaLoading™ - 4x 0.5 ml, for loading the samples into the wells in the agarose gel

Not included with the kit:

0.5-ml clear microcentrifuge tube (1 for each student)

20 µl adjustable Pipettor (1 for each group of 6 students, ~\$100 each)

20 µl pipette tips (1 box with 980 tips each box)

2000-3000 rpm minicentrifuge (1 for each group of 6 students, ~\$200 each)

Mini vortex mixer (1 for each group of 6 students, ~\$200 each)

Deionized water (4x500 ml, requested at your local research labs)

Agarose powder (100 g, purchased from scientific supply stores)

TBE buffer (100 g, pre-mix powder, purchased from scientific supply stores)

Mini electrophoresis unit (1 for each group of 12 students, ~\$500 each)

Ideas for lab curriculum extensions

- Try using deionized water or bottled water as electrophoresis buffer
- Discuss pH, electrolysis, electrical currents, potentials, and conductivity
- Run 4 food colors with the electrophoresis to explain how DNA sequencer works
- Discuss the precautions of staining DNA/RNA with ethidium bromide
- Hold a DNA/RNA lab summer camp for k-12 kids in your city

Protocol For Running Agarose Gel Electrophoresis

Gel electrophoresis is a commonly used method to separate DNA, RNA, and protein molecules. At the pH used in electrophoresis these molecules remain negative, the speed of their migration in the gel matrix towards the cathode is based on their size – the smaller the molecule the faster it moves through the gel.

1. Cast agarose gel (this step may be performed by your teacher before the class)

- 1) Weigh out 3 g of agarose powder and place it in a 500-ml glass bottle. Add 400 ml of TBE buffer to the agarose (0.7% agarose) and swirl the bottle to mix well.
- 2) Heat the agarose suspension (leave the lid open!) in a microwave oven for 2-3 minutes to dissolve the agarose. Remove the bottle (wear heat-resistant mittens!) from the oven and slowly swirl the agarose solution (BE CAREFUL AND SLOW, the solution may be superheated and could spill out!) to see if the agarose particles have gone into solution.
- 3) Repeat the heating for 1-2 minutes until all agarose particles have disappeared.
- 4) Cool down the agarose solution in a fridge for ~10 minutes to lukewarm and pour the solution (~50 ml for each 12-lane mini gel cassette) into a gel cassette.
- 5) Place TWO same size combs at each end of the gel cassette with the two combs lined up well and separated by 1 inch (2.54 cm).
- 6) Leave the cassette in the fridge for ~20 minutes until the gel becomes solidified.

2. Load the samples and AquaStain™

- 1) Vortex the DNA/RNA sample prepared in previous lab. Centrifuge the tube for 4 minutes to pellet the insoluble.
- 2) Take 5 µl of your DNA/RNA solution and mix with 5 µl of AquaLoading™ dye in a 0.5-ml microfuge tube.
- 3) Remove the gel combs and put the agarose gel cassette in the electrophoresis chamber. Fill the chamber with TBE buffer to cover the gel.
- 4) Load 10 µl of AquaStain™ dye to each well at one end of the gel (the cathode side!) and load each DNA/RNA sample in a well at the other end of the gel (the anode side!).

3. Run the electrophoresis

- 1) Turn on the power supply and adjust the electrophoresis voltage to about 100 V. You should start to see the orange AquaLoading™ dye moving towards the cathode while the blue AquaStain™ dye moving towards the anode.
- 2) Continue the electrophoresis for ~45 minutes until the positively charged AquaStain™ dye meets, binds and stains the negatively charged DNA in the gel, where the DNA and RNA appear as dark blue bands (the DNA band will fade away if the electrophoresis is continued).