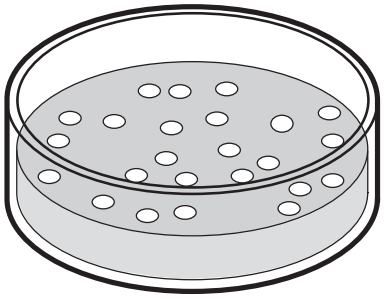


Bio-Rad Explorer

Microbial Culturing Kit

Catalog #1665020EDU

explorer.bio-rad.com

Store components of this kit at room temperature.

BIO-RAD

DIR ID 10000098470/Z05/000/C 2018-09-13 Released CM# 500000019391

Table of Contents

Introduction

The microbial culturing kit provides the materials and methods for a wide variety of microbiology activities. The kit can be used to make both liquid and solid media suitable for the growth of small or large cultures of either environmental microbes or laboratory strains of *E. coli.*

The Microbial Culturing Kit is also part of the Bio-Rad Cloning and Sequencing Explorer Series (catalog #1665000EDU) and contains the materials used in the Bio-Rad Microbes and Health Kit (catalog #1665030EDU).

For more microbiology curricula visit explorer.bio-rad.com and look for the pGLO Bacterial Transformation Kit (catalog #1660003EDU) or the Microbes and Health Kit.

Kit Inventory Checklist

Kit Components	Number/Kit	(✔)
Ampicillin	2 vials	❒
LB nutrient agar powder (to make 500 ml)	1 pouch	❒
Petri dishes, sterile, bag of 20	2 bags	❒
Culture tubes, 60 mm, sterile, bag of 25	3 bags	❒
Inoculation loops, sterile, 10 µl, pack of 10 loops	6 pks	❒
E. coli HB101 K-12, lyophilized	1 vial	❒
LB broth capsules, bag of 12 (to make 50 ml each)	1 bag	❒
Disposable plastic transfer pipets (DPTPs), pack of 10	1 pack	❒

Refills Available Separately

Microbial Culturing Kit Refill Package (catalog #1665021EDU), contains ampicillin, LB broth capsules, LB agar, and *E. coli* strain HB101 K-12

LB nutrient agar powder (catalog #1660600EDU), 1 pkg

Ampicillin (catalog #1660407EDU), 1 vial

E. coli strain HB101 K-12 (catalog #1660408EDU), 1 vial

Recommended Accessories

Microwave, autoclave, or heated magnetic stir plate

Glass flasks

Graduated cylinder

Thermometer

Incubation oven

Safety Issues

Ampicillin may cause allergic reactions or irritation to the eyes, respiratory system, and skin. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing. Ampicillin is a member of the penicillin family of antibiotics. Those with allergies to penicillin or any other member of the penicillin family of antibiotics should avoid contact with ampicillin.

The host organism in this kit, an *E. coli* K-12 strain, is not pathogenic. However, handling of *E. coli* K-12 requires the use of Standard Microbiological Practices. These practices include but are not limited to the following. Work surfaces are decontaminated once a day and after any spill of viable material. All contaminated liquid or solid wastes are decontaminated before disposal. Persons wash their hands: (i) after they handle materials involving organisms containing recombinant DNA molecules, and (ii) before exiting the laboratory. All procedures are performed carefully to minimize the creation of aerosols. Mechanical pipetting devices are used; mouth pipetting is prohibited. Eating, drinking, smoking, and applying cosmetics are not permitted in the work area. Wearing protective eyewear and gloves is strongly recommended.

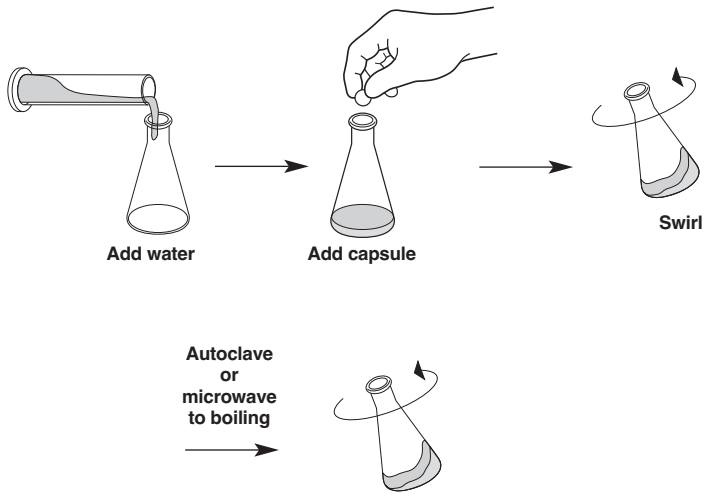
Preparation of Liquid Media

Preparation of Ampicillin

Ampicillin is an antibiotic used to selectively eliminate bacteria that have not been transformed with plasmids containing an ampicillin resistance gene. Ampicillin is shipped freeze dried in a small vial containing 30 mg of the antibiotic. With a sterile pipet, add 3 ml of sterile water directly to the vial to rehydrate the antibiotic to make a 10 mg/ml or 200x solution. Ampicillin is used at a final concentration of 50 µg/ml in both LB-ampicillin broth and LB-ampicillin agar. Ampicillin should be stored at –20°C and is good for 1 year.

Preparation of LB Broth

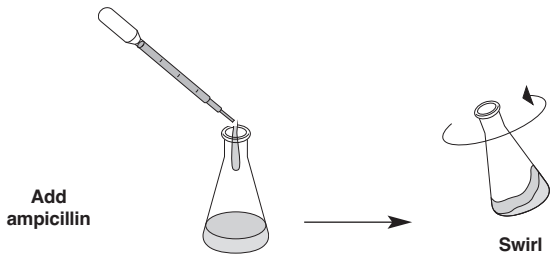
This protocol is used to prepare liquid LB broth for growth of bacteria. LB broth has been supplied in convenient capsule form; each capsule makes 50 ml of LB broth when reconstituted with water. To avoid contamination of a common stock of LB broth, it is recommended that each capsule be reconstituted with 50 ml of water in a separate container. To make 50 ml of sterile LB broth, label a clean container and add one capsule of LB and 50 ml of distilled water and cover appropriately. (**Note**: never completely seal a container that is to be autoclaved.) Allow the capsule to dissolve. Swirling or stirring with a magnetic stir bar will hasten this process. Autoclave the container on the wet cycle for 30 min. Allow the broth to cool to room temperature before use. If an autoclave is not available, LB can be filter sterilized through a 0.2 µm filter, or can be sterilized in the microwave by heating to boiling at least three times. (Note: reduce evaporation by using a reduced power setting on the microwave so the solution simmers rather than boils.)



Storage of LB broth at 4°C is recommended. The broth can be stored for up to 1 year. LB broth can also be stored at room temp, however this is not recommended if the microwave method has been used for sterilization or if the bottle has been opened after sterilization. LB broth containing antibiotics should be stored at 4°C and is good for one month.

Preparation of Ampicillin Broth

This protocol is used to prepare liquid LB broth with ampicillin, which will select for the growth of bacteria transformed with plasmids containing an ampicillin resistance gene. The final concentration of ampicillin in LB-ampicillin broth should be 50 µg/ml. To make 25 ml of LB broth containing 50 µg/ml ampicillin, add 125 µl of 10 mg/ml ampicillin stock. Ensure LB broth is cooled to room temperature before adding ampicillin. Excessive heat will degrade the antibiotic. LB broth containing antibiotics should be stored at 4°C and is good for one month.



Starting Minipreps

This protocol is used to grow small cultures of bacteria in LB broth with ampicillin, which will select for the growth of bacteria transformed with plasmids containing an ampicillin resistance gene. The small cultures are often processed to isolate plasmid DNA for experiments and activities in molecular biology.

1. Prepare LB-ampicillin broth as described above.
2. Label an appropriate number of 15 ml culture tubes.
3. Using sterile technique, pipet 5 ml of LB-ampicillin broth into each 15 ml culture tube.
4. One day prior to the next lab session, use a sterile pipet tip or sterile loop to pick a single colony and inoculate an LB-ampicillin culture tube. A sterile pipet tip is often better than a sterile loop to pick single colonies. Repeat for each miniprep culture from individual colonies.

Note: Occasionally satellite colonies may grow, so it is important to pick the large individual colonies instead of the tiny colonies surrounding larger colonies. Be sure that a single colony is picked, otherwise you may isolate multiple plasmids from your miniprep and these cannot be sequenced.

5. Place the miniprep cultures to grow in a shaking incubator or water bath at 37°C overnight. The liquid cell culture should be shaken vigorously to provide a sufficient amount of oxygen to the dividing cells. More vigorous shaking is better. Cells often may be grown at room temperature with shaking but will require more time to grow.
6. Larger cultures can be inoculated by transferring 10 µl from the miniprep into a sterile flask containing LB-ampicillin and shaking.
7. Plasmid DNA can be isolated from minipreps using a plasmid isolation protocol such as the Aurum Plasmid Mini Kit (catalog #7326400EDU).

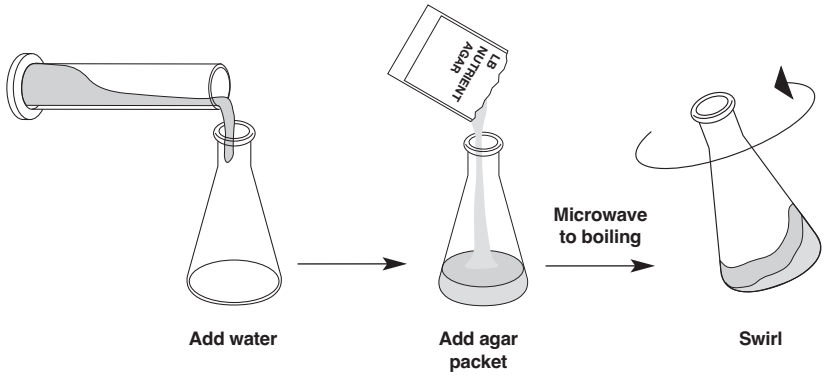
Preparation of Solid Media

Preparation of Ampicillin

Ampicillin is an antibiotic used to selectively eliminate bacteria that have not been transformed with plasmids containing an ampicillin resistance gene. Ampicillin is shipped freeze dried in a small vial containing 30 mg of the antibiotic. With a sterile pipet, add 3 ml of sterile water directly to the vial to rehydrate ampicillin to make a 10 mg/ml or 200x solution. Ampicillin is used at a final concentration of 50 µg/ml in both LB-ampicillin broth and LB-ampicillin agar. Ampicillin should be stored at –20°C and is good for 1 year.

Preparation of LB Agar

This protocol is used to prepare solid LB agar media for the growth of bacteria. Ideally agar plates should be prepared at least two days before required and left out at room temperature for two days and then refrigerated until they are to be used. Two days on the benchtop allows the agar to dry out and more readily take up the liquid transformation solution.



To prepare 500 ml LB agar, add the entire contents of the LB nutrient agar packet to 500 ml of distilled water in a 1 L or larger Erlenmeyer flask. Swirl to dissolve the agar, or add a magnetic stir bar to the flask and stir on a stir plate. A stir bar will also aid in mixing the solution completely once sterilization is complete. Autoclave LB agar on wet cycle for 30 min. Once the autoclave cycle is complete, check the solution to ensure that the agar is all dissolved. If no autoclave is available, heat the agar solution to boiling in a microwave. Repeat heating and swirling about three times using a reduced power setting on the microwave — to reduce evaporation and boiling over — until all the agar is dissolved (i.e., no more specks swirl around).

Be sure to wear appropriate protective equipment and be careful to allow the flask to cool slightly before swirling so that the hot medium does not boil over onto your hand. When the agar is dissolved, allow the LB nutrient agar to cool so that the outside of the flask is just comfortable to hold (~50°C). A water bath set at 50°C is useful for this step. While the agar is cooling, label the plates. Be careful not to let the agar cool so much that it begins to solidify.

Preparation of LB-Ampicillin Agar

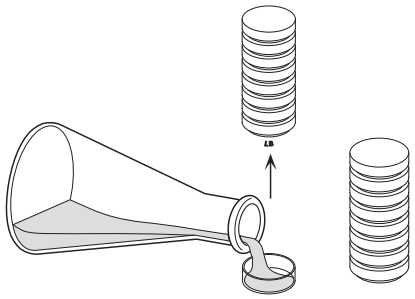
This protocol is used to prepare solid LB-ampicillin media, which will select for the growth of bacteria transformed with plasmids containing an ampicillin resistance gene. The final concentration of ampicillin in LB-ampicillin agar should be 50 µg/ml. To make 500 ml of LB agar containing 50 µg/ml ampicillin, add 2.5 ml of 10 mg/ml ampicillin stock. Ensure LB agar is cooled to 50°C before adding ampicillin. Excessive heat will degrade the ampicillin. Swirl or use the stir bar and a stir plate to mix the ampicillin into the agar, taking care not to introduce bubbles.

Preparation of LB-Ampicillin IPTG Agar

This protocol is used to prepare solid LB-ampicillin IPTG (isopropyl b-D-1-thiogalactopyranoside) media, which will select for the growth of bacteria transformed with plasmids containing an ampicillin resistance gene. IPTG is used to increase protein expression induced by the lac operon. The final concentration of ampicillin in LB-ampicillin IPTG agar should be 50 µg/ml and the final concentration of IPTG should be 0.2 mM. To make 500 ml of LB broth containing 50 µg/ml ampicillin and 0.2 mM IPTG, add 2.5 ml of 10 mg/ml ampicillin stock and 100 µl of 1 M IPTG. Ensure LB agar is cooled to 50°C before adding ampicillin and IPTG; excessive heat will degrade the reagents. Swirl or use the stir bar and a stir plate to mix the ampicillin and IPTG into the agar taking care not to introduce bubbles. IPTG is not included in this kit. A 1 M stock of IPTG is provided in Bio-Rad's Ligation and Transformation Module (#1665015EDU). Agar plates containing IPTG are required to select colonies using the Ligation and Transformation Module.

Procedure for Pouring Plates

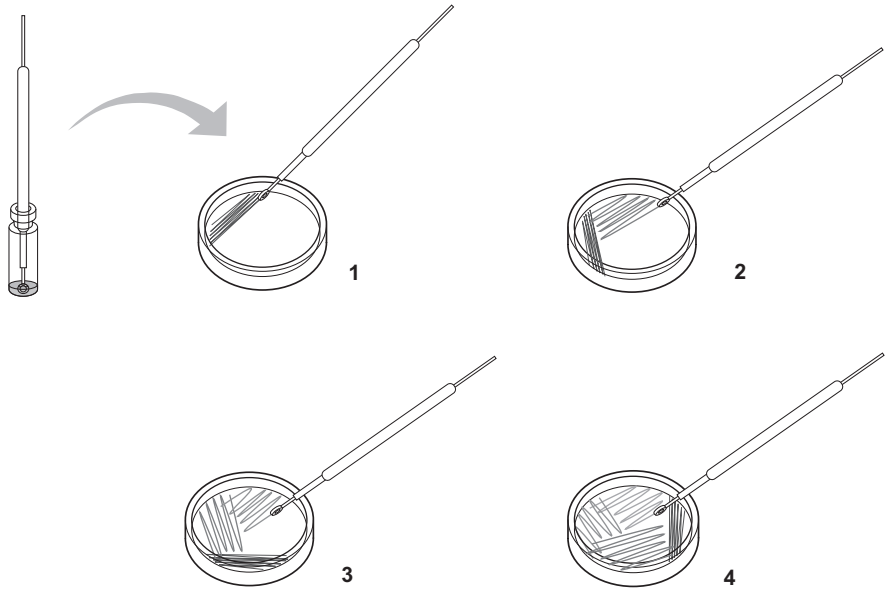
This protocol is used to prepare petri plates using the solid LB agar, LB-ampicillin agar or LB-ampicillin IPTG agar media. Label the outside of the lower plate rather than the lid of the plates that are to be prepared. Stack empty plates 4–8 high and, starting with the bottom plate, lift the lid and the upper plates straight up and to the side with one hand and pour the LB agar with the other. Fill the plate about one third to one half (~10 ml) with agar, replace the lid and continue up the stack. Let the plates cool and solidify in this stacked configuration; do not disturb them until the agar has solidified. Wipe any agar drips off the sides of the plates. LB, LB-ampicillin, and LB-ampicillin IPTG agar plates should be stored at 4°C enclosed in plastic bags or plastic wrap to avoid drying out and are good for one month.



Rehydrate Bacteria and Streak Plates

Streaking is done to make single colonies from concentrated bacteria. Each colony grows from one bacterium and thus a colony is a "clone," or group of genetically identical individuals. A tiny drop of the original bacterial suspension contains millions or billions of individual bacteria and must be diluted multiple times to isolate single bacteria. Under favorable conditions *E. coli* can double every 20 minutes and thus a single bacterium will multiply to become billions of genetically identical cells in less than 24 hours.

1. Using a sterile pipet, rehydrate the lyophilized *E. coli* HB101 by adding 250 µl of LB nutrient broth directly to the vial. Recap the vial and shake it gently to ensure all bacteria are rehydrated. Incubate the vial at 37°C for 8–24 hr. If an incubator or water bath is not available, the vial can be placed into a large volume of water heated to 37°C and then left at room temperature for 16–24 hr. Store remaining LB broth at 4°C until use.
2. Insert a sterile inoculation loop into a bacterial colony or other sample. Insert the loop straight into the container without tilting. Remove the loop and gently rub it back and forth over the agar in the top left hand corner as shown below. The first streak dilutes the cells. Go back and forth with the loop about a dozen times in the first quadrant. Do not break the surface of the agar.



3. For subsequent streaks, the goal is to use as much of the surface area of the plate as possible. Rotate the plate about 45 degrees (so that the streaking motion is comfortable for your hand) and start the second streak. Do not dip the loop into the starting material (bacterial colony, rehydrated bacteria, or other sample) again. Go into the previous streak one or two times and then back and forth as shown about a dozen times.

In subsequent quadrants the cells become more and more dilute, increasing the likelihood of producing single colonies. Remember a single colony arose from one cell and all the cells in the colony are genetically identical.

4. Rotate the plate again and repeat streaking into the third quadrant.
5. Rotate the plate again and make the final streak. **Do not touch the first quadrant**.
6. Place the plates upside down inside the incubator for 16–24 hours at 37°C.

Glossary

Agar — Agar is a jelly-like substance obtained from seaweed. It is made of linked sugars (a polysaccharide) and is used to make medium for growing bacteria.

Ampicillin — Ampicillin is a penicillin-like bactericidal antibiotic that inhibits the synthesis of the peptidoglycan component of bacterial cell walls, especially in gram-positive bacteria but also in some gram-negative bacteria such as *E. coli*.

Antibiotic — An antibiotic is a chemical that prevents or reduces the growth of bacteria or other microbes.

Bacteria — Bacteria are single-cell microorganisms with no nucleus.

Bactericidal — An antibiotic or other agent that kills bacteria is termed bactericidal.

Bacteriostatic — An antibiotic or other agent that prevents the growth of bacteria is termed bacteriostatic.

Binary fission — Asexual reproduction achieved by duplicating DNA and dividing into two halves. Most bacteria reproduce this way.

Clone — Clones are genetically identical organisms.

Colony — A bacterial colony is a group of bacteria on growth media that usually have grown from a single bacterium. A colony is thus a clone of identical organisms.

E. coli — *Escherichia coli* is a gram-negative facultative anaerobic bacillus bacterium. It inhabits the intestines of animals and humans and may benefit them by producing vitamin K and preventing the spread of harmful bacteria. Harmless, genetically weakened forms of *E. coli* such as the HB101 strain used in this kit are used in many scientific applications. Normally *E. coli* is harmless but a few strains, such as O157:H7, can cause disease.

LB — Luria Bertani broth (sometimes called Lysogeny broth) is composed of yeast extract, tryptone, and sodium chloride and is commonly used to culture bacteria.

Penicillin — Penicillin is a bactericidal antibiotic that inhibits the synthesis of the peptidoglycan component of bacterial cell walls, especially in gram-positive bacteria. Penicillin was discovered by Alexander Fleming in 1928 and was the first antibiotic to be used medically.

Petri dish — Petri dishes are small round, flat containers made of glass or plastic. They are commonly used to hold media used to culture microbes. Petri dishes were invented by microbiologist Julius Petri, an assistant to Robert Koch.

(01)03610520611821

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Bio-Rad Laboratories, Inc.

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Web site bio-rad.com **USA** 1 800 424 6723 **Australia** 61 2 9914 2800 **Austria** 43 01 877 89019 **Belgium** 32 03 710 53 00 **Brazil** 55 11 3065 7550
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10000098470 Ver C (1665022) US/EG 0718 Sig 0118

SIGNATURE PAGE

Document: 10000098470/Z05/000/C

Document Description: MS1665022 MANUAL, MICROBIAL CULTURING

Engineering Record Number: 200105608

Engineering Record Description: [925-CR-064] Manual, Microbial Culturing

Change Master Number: 500000019391

Signature List:

Date	Time (PT)	User	Signature on Status
27 August 2018	10:24:09	Ingrid L Hermanson-Miller	Approved