Refresh Kit Components — Large Class/Multiple Class Preparation Guide

Biotechnology Explorer™ kits include reagents in quantities sufficient for a classroom with 8–12 workstations and 4 students per workstation, but what if you have a larger class or multiple class sections? To enable the kits to be used for large or multiple classes, Bio-Rad Laboratories offers refill packages of many of the consumables. These are designated as Refresh Kit Components in our catalog and on our website (explorer.bio-rad.com). This guide describes how to prepare LB agar plates and agarose gels on a large scale using these Refresh Kit Components to accommodate large or multiple classes.

Agar Plate Preparation

LB agar plates are used in several Biotechnology Explorer kits, including:

- pGLO™ Bacterial Transformation kit (catalog #166-0003EDU)
- Secrets of the Rainforest™ kit (catalog #166-0006EDU)
- Microbes and Health kit (catalog #166-5030EDU)

1. Number of Agar Plates per Workstation

Each kit requires a different number of plates and different types of plate per workstation. The number of plates of each type needed for one and for multiple workstations is shown in Table 1. It is recommended that LB agar plates be prepared at least two days before they are needed to provide time for the plates to dry adequately.

Table 1. Number of 60 mm LB agar plates required for specified number of workstations.

Number of Workstations	1	8	16	32	64	128	256	512
Number of Kits	1	1	2	4	8	16	32	64
pGLO Bacterial Transformation Kit								
LB agar plates	2	16	32	64	128	256	512	1,024
LB ampicillin agar plates	2	16	32	64	128	256	512	1,024
LB ampicillin arabinose agar plates	1	8	16	32	64	128	256	512
Secrets of the Rainforest Kit								
LB ampicillin arabinose agar plates	2	16	32	64	128	256	512	1,024
Microbes and Health Kit								
LB sugar agar plates	3	24	48	96	192	384	768	1,536

2. Formulation of LB Agar

The concentrations of the reagents needed to prepare LB agar plates are provided in Table 2.

Note: The protocols provided here give exact concentrations of ampicillin and arabinose. These concentrations may differ from those recommended in the instruction manual for each particular kit. Both ampicillin and arabinose function at a range of concentrations; either set of directions can be used successfully.

- LB nutrient agar is available from Bio-Rad as a powder in 20 g and 500 g sizes. LB agar powder is added directly to purified water, stirred or swirled until the powder goes into solution, and then heat-sterilized
- Freeze-dried ampicillin is available from Bio-Rad in 30 mg vials. Vials should be rehydrated with 3 ml of sterile water, transformation solution, or TE (Tris-HCl EDTA) buffer to make a 200x stock solution of 10 mg/ml. If ampicillin is made from ampicillin powder purchased from a different source, the solution must be filter-sterilized before use
- Freeze-dried arabinose is available from Bio-Rad in 600 mg vials. Vials should be rehydrated with 3 ml of sterile water, transformation solution, or TE buffer to make a 100x stock solution of 200 mg/ml. If arabinose is made from arabinose powder purchased from a different source, the solution must be filter-sterilized before use
- Sugar or sucrose is added directly to the LB agar powder and heat-sterilized along with the LB agar

Table 2. Concentrations of reagents and stock solutions.

Formulation	Final Concentration in Molten LB Agar	Stock Solution Preparation
LB agar	Tryptone (10 g/L), yeast extract (5 g/L), sodium chloride (5 g/L), agar (15 g/L)	Add LB agar powder directly to purified water prior to sterilization
Ampicillin*	50 mg/L	**200x stock solution: 10 mg/ml** Add 3 ml of sterile water, transformation solution, or TE to 30 mg of lyophilized ampicillin (catalog #166-0407EDU). Store at –20°C for ≤1 yr.
Arabinose*	2 g/L	**100x stock solution: 200 mg/ml** Add 3 ml of sterile water, transformation solution, or TE to 600 mg of lyophilized arabinose (catalog #166-0406EDU). Mix the vial; wait at least 10 min for arabinose to dissolve. Store at –20°C for ≤1 yr.
Sugar	10 g/L	Add table sugar or sucrose directly to LB agar powder prior to sterilization.

* Sensitive to heat — add only when molten LB agar is at ~50°C.

3. Volume of LB Agar to Prepare

Each 60 mm petri dish requires 7–10 ml of molten agar. Table 3 is based on 10 ml of agar prepared per plate, but more plates can be made if 7–9 ml of molten agar is used per plate. The required amount of each reagent is shown in the table for a variety of final volumes of agar.

A 2 L flask should be used to prepare only 1–1.5 L of agar, not 2 L — if the flask is full, it will boil over when sterilized. Multiple flasks can be sterilized at one time, depending on the size of the autoclave or microwave oven. The mix in each flask should be prepared separately; do not make one big batch and divide it into the flasks.

Table 3. Volumes and quantities of reagents for LB agar plates.

Number of 60 mm Plates Required Volume of LB Agar Required	~20 200 ml	~50 500 ml	~100 1 L	~150 1.5 L	~250 2.5 L	~500 5 L	~1,000 10 L
LB Agar Plates							
Purified water, ml	200	500	1,000	1,500	2,500	5,000	10,000
LB agar, g	7	17.5	35	52.5	87.5	175	350
LB Ampicillin Agar Plates							
Purified water, ml	200	500	1,000	1,500	2,500	5,000	10,000
LB agar, g	7	17.5	35	52.5	87.5	175	350
Ampicillin* 10 mg/ml stock, ml	1	2.5	5	7.5	12.5	25	50
LB Ampicillin Arabinose Agar Plates							
Purified water, ml	200	500	1,000	1,500	2,500	5,000	10,000
LB agar, g	7	17.5	35	52.5	87.5	175	350
Ampicillin* 10 mg/ml stock, ml	1	2.5	5	7.5	12.5	25	50
Arabinose* 200 mg/ml stock, ml	2	5	10	15	25	50	100
LB Sugar Agar Plates							
Purified water, ml	200	500	1,000	1,500	2,500	5,000	10,000
LB agar, g	7	17.5	35	52.5	87.5	175	350
Sugar, g	2	5	10	15	25	50	100
Number of Vials							
Ampicillin* (30 mg)	1	1	2	3	5	9	17
Arabinose* (600 mg)	1	2	4	5	9	17	34

* Sensitive to heat — add only when molten LB agar has cooled down to ~50°C.

4. Preparation of LB Agar Plates

- Add the required quantity of LB agar powder (and sugar, if needed) to purified or distilled water (see Table 3) in an Erlenmeyer flask
- Swirl to dissolve the LB powder, or place a magnetic stirbar in the flask and stir on a stir plate (Note: Agar will not dissolve until it is heated). A stirbar will also aid in mixing the solution completely once sterilization is complete
- Autoclave LB agar on wet cycle for 20 min per liter of agar (Note: Never autoclave a bottle with a secured cap — caps must always be loose to allow steam to escape). Once the autoclave cycle is complete, check the solution to ensure that the agar is totally dissolved
- If an autoclave is not available, heat the agar solution to boiling in a microwave oven with the flask loosely covered — never heat with the cap secured. (Note: Inserting a 25–50 ml Erlenmeyer flask upside down into the opening of the 2 L flask while microwaving will create a reflux chamber that helps to reduce evaporation)
- Repeat heating and swirling three times. Use a low power setting on the microwave oven to reduce evaporation and prevent boiling over until all the agar is dissolved (clear specks are no longer seen). Wear appropriate protective equipment; let the flask cool slightly before swirling so that the hot medium does not boil over onto your hand

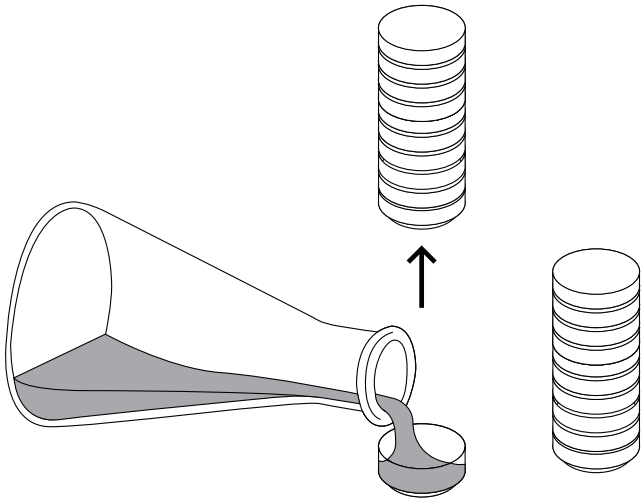
Caution: Always wear heat-protective gloves, goggles, and a lab coat while preparing and casting agar plates. Hot molten agar and the flasks containing hot agar can cause severe burns.

- When the agar is dissolved, allow the LB nutrient agar to cool just until the outside of the flask is 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
- If ampicillin or arabinose is required, add the required volume of sterile stock solution to the 50°C molten agar and gently swirl (or use the sterilized stir bar) to mix; take care not to introduce bubbles into the solution

5. Pouring Plates

Ideally, agar plates should be prepared at least two days before they will be needed, left out at room temperature for two days, and 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 solution upon inoculation

- Label the outside of the lower plate, not the lid
- Gently swirl the agar to mix prior to pouring
- Stack empty plates 4–8 high. 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 hand (see illustration)



- Fill the plate one third to one half full (~10 ml) with agar, replace the lid, and continue up the stack. Alternatively, a 10–50 ml sterile pipet with a pipet controller may be used to aliquot 7–10 ml of molten LB agar into each petri dish
- Let the plates cool and solidify while stacked; do not disturb them until the agar has solidified. Wipe any dripped agar off the sides of the plates
- Leave plates at room temperature for 2 days so that excess water evaporates
- Wrap plates in plastic and store at 4°C for up to 1 month

Agarose Gel Preparation

Agarose gels are used in several Biotechnology Explorer DNA electrophoresis kits.

Kits requiring 1% TAE (Tris-acetate-EDTA) agarose gels:

- Analysis of Precut Lambda DNA kit (catalog #166-0001EDU)
- Restriction Digestion and Analysis of Lambda DNA kit (catalog #166-0002EDU)
- Forensic DNA Fingerprinting kit (catalog #166-0007EDU)
- PV92 PCR Informatics kit (catalog #166-2100EDU)
- Cloning and Sequencing Explorer Series, *GAPDH* PCR module (catalog #166-5010EDU)

Kits requiring 3% TAE agarose gels:

- Crime Scene Investigator PCR Basics™ kit (catalog #166-2600EDU)
- GMO Investigator™ kit (catalog #166-2500EDU)

The kits require either one 7 x 7 cm gel with one 8-well comb for one workstation or one 7 x 10 cm gel with two 8-well combs for two workstations. (Note: The *GAPDH* PCR module requires either one gel with 12 wells or two gels per group). The agarose gels required for the kits are either 1% or 3% agarose gels depending on the application (higher percentage agarose separates smaller DNA fragments more effectively).

The following protocol describes how to prepare electrophoresis buffer and large numbers of agarose gels.

Table 4. Volumes and quantities of reagents for agarose gels.

Number of Gels*	1	4	8	16	32	64	128
1% TAE Agarose Gel (7 x 7 cm) — serves one workstation							
Purified water, ml	39	157	314	627	1,254	2,509	5,018
50x TAE, ml**	0.8	3.2	6.4	12.8	25.6	51.2	102.4
Agarose, g	0.4	1.6	3.2	6.4	13	26	51
TOTAL volume of molten agarose, ml	40	160	320	640	1,280	2,560	5,120
1% TAE Agarose Gels (7 x 10 cm) — serves two workstations							
Purified water, ml	49	196	392	784	1,568	3,136	6,272
50x TAE, ml	1.0	4.0	8.0	16	32	64	128
Agarose, g	0.5	2.0	4.0	8.0	16	32	64
TOTAL volume of molten agarose, ml	50	200	400	800	1,600	3,200	6,400
3% TAE Agarose Gels (7 x 7 cm) — serves one workstation							
Purified water, ml	39	157	314	627	1,254	2,509	5,018
50x TAE, ml**	0.8	3.2	6.4	12.8	25.6	51.2	102.4
Agarose, g	1.2	4.8	10	19	38	77	154
TOTAL volume of molten agarose, ml	40	160	320	640	1,280	2,560	5,120
3% TAE Agarose Gels (7 x 10 cm) — serves two workstations							
Purified water, ml	49	196	392	784	1,568	3,136	6,272
50x TAE, ml	1.0	4.0	8.0	16	32	64	128
Agarose, g	1.5	6.0	12	24	48	96	192
TOTAL volume of molten agarose, ml	50	200	400	800	1,600	3,200	6,400

* It is advisable to pour a few more gels than you need as a backup in case students break or damage their gels.
** For ease of pipetting, volumes of 50x TAE larger than 10 ml can be rounded to the nearest ml.

1. Preparation of Agarose Gels

See Table 4 for the quantities of reagents for your application.

- Add the agarose powder to a suitable container (if using a microwave oven, ensure that the container fits in the oven); fill to less than 75% of the volume of the container
- Add the required volume of purified water and 50x TAE buffer; swirl to suspend the agarose powder in the buffer
    - If you are using an Erlenmeyer flask, invert a small 25 ml flask into the open end of the larger flask. The small flask acts as a redux chamber that allows for long or vigorous boiling without much evaporation
    - If you are using a bottle, it is very important to loosen the cap so that air and steam can escape from the bottle
    - Tip: Mark the flask or bottle at the level of the solution. If boiling causes a large loss of volume (which would change the percentage of agarose), the original volume can be regained by adding hot purified water to bring the solution level back to the original mark

Caution: Always wear heat-protective gloves, goggles, and a lab coat while preparing and casting agarose gels. Hot molten agarose and the flasks containing hot agarose can cause severe burns.

- Use a medium power setting and an appropriate time setting depending on the power of your microwave oven and the volume of liquid. If you don't know how long the solution will take to boil, heat the solution for 2 min, swirl the flask, and continue to heat in 2 min increments until the solution boils
- When the solution boils, heat for 30 sec increments and swirl the flask after each until all of the small transparent agarose particles are dissolved. If a microwave oven is not available, a magnetic hot plate can be used with a stirbar in the agarose solution. Boil the solution until the transparent particles are dissolved; do not let the solution boil over
- Add hot purified water to restore original level of mixture if needed
- Allow the agarose to cool to 50–60°C (a water bath can be used for this step) prior to casting gels

2. Casting Gels

There are a variety of ways to cast agarose gels. This section outlines the tape-the-tray method for casting gels. Other methods are detailed in the Sub-Cell® GT cell (electrophoresis chamber) instruction manual (bulletin M1704400).

- Seal the ends of the gel tray securely with strips of standard laboratory tape (not regular sticky tape). Press the tape firmly to the edges of the gel tray to form a fluid-tight seal. Level the gel tray on a leveling table or workbench using the leveling bubble provided with the chamber
- While the agarose is cooling to 60°C, place the comb into the appropriate slot of the gel tray. Gel combs should be placed within a half inch of the end of the gel casting tray if a single-well, 7 x 7 cm gel is cast. To pour a double-well gel using a 7 x 10 cm tray and two 8-well combs, place one comb at one end of the tray and the other comb in the middle of the tray. The combs will form the wells into which the samples will be loaded
- Pour enough agarose to cover the gel comb teeth or to a depth of 0.5–0.75 cm
- Allow the gel to solidify at room temperature for 10–20 min. Do not move or handle the gel tray until the gel has solidified. It will appear cloudy or opaque when it is ready to use
- Carefully remove the comb from the solidified gel taking care not to tear the wells
- Store gels in a sealable plastic bag at room temperature for up to 1 day or in the refrigerator for up to 1 week

Table 5. Volumes and quantities of reagents for electrophoresis buffer.

Number of Electrophoresis Chambers	1	4	8	16	32	64	128
1x TAE Buffer							
Purified water, ml	270	1,078	2,156	4,312	8,624	17,248	34,496
50x TAE, ml	5.5	22	44	88	176	352	704
Total volume of 1x TAE buffer, ml	275	1,100	2,200	4,400	8,800	17,600	35,200
0.25x TAE Buffer							
Purified water, ml	274	1,094	2,189	4,378	8,755	17,510	35,021
50x TAE, ml	1.4	5.6	11	22	45	90	179
Total volume of 0.25x TAE buffer, ml	275	1,100	2,200	4,400	8,800	17,600	35,200

** See fast gel protocol (bulletin 5396)

3. Preparation of TAE Buffer

The conventional way to electrophorese DNA with TAE electrophoresis buffer is to use TAE at a 1x concentration for both gel casting and running buffer. All Biotechnology Explorer DNA electrophoresis kits specify that the gels are electrophoresed at 100 V for 30 min. A fast gel protocol (bulletin 5396) was developed by scientists at Bio-Rad to shorten the length of time required to run agarose gels to 20 min by using a lower concentration (0.25x) of TAE running buffer and a higher voltage (200 V). Download a copy of the protocol from the Bio-Rad website for reference.

Note: Never run a gel using 1x TAE at 200 V; the buffer will heat up and your gel will melt.

To prepare either 1x or 0.25x TAE buffer, combine purified or distilled water with the required amount of 50x TAE buffer (see Table 5) and mix well.

1x TAE buffer can be reused 4–5 times and 0.25x TAE can be reused 2–3 times. However, if you are using gel tanks sequentially with different classes, ensure that the buffer is at or below room temperature before reusing it. If the buffer is already warm, it may become hot enough to melt the agarose gel during the run, especially if you are using 200 V with the 0.25x buffer.

4. Electrophoresis of Samples

To electrophorese the samples, place the 1x TAE agarose gel into an electrophoresis chamber and cover the gel in TAE buffer to 1–2 mm above the surface of the gel. Load the DNA samples into the wells of the gel and electrophorese at the recommended voltage for the specified time period.

Ordering Information

Catalog #	Product Description
166-0555EDU	pGLO Bacterial Transformation Kit Refill Package
166-0405EDU	pGLO Plasmid, 20 µg, lyophilized
166-0406EDU	Arabinose, 600 mg, lyophilized
166-0407EDU	Ampicillin, 30 mg, lyophilized
166-0408EDU	*E.coli* Strain HB101 K-12, lyophilized
166-0409EDU	Transformation Solution, 15 ml
166-0421EDU	LB Broth, 10 ml
166-0600EDU	LB Nutrient Agar Powder, 20 g, makes forty 60 mm agar plates
166-0472EDU	LB Nutrient Agar Powder, 500 g, makes one thousand 60 mm agar plates
166-0470EDU	Petri Dishes, 60 mm, sterile, 500
166-0471EDU	Inoculation Loops, 10 µl, sterile, 80
166-0479EDU	Jellyfish Foam Floating Racks, 8 racks with 12 microcentrifuge tube wells
166-0530EDU	Long-Wave UV Pen Light, 1
166-0500EDU	Long-Wave UV Lamp, 1
166-5009EDU	Master Mix for PCR, 2x, 90 units, 1.2 ml
166-0742EDU	50x TAE, 100 ml
161-0743EDU	50x TAE, 1 L
161-0773EDU	50x TAE, 5 L cube
161-0767EDU	Sample Loading Dye, 5x, 10 ml
161-3100EDU	Certified™ Molecular Biology Agarose, 25 g
161-3101EDU	Certified Molecular Biology Agarose, 125 g
161-3102EDU	Certified Molecular Biology Agarose, 500 g
166-0420EDU	Fast Blast™ DNA Stain, 500x, 100 ml
166-0450EDU	Small Fast Blast DNA Electrophoresis Reagent Pack
166-0455EDU	Medium Fast Blast DNA Electrophoresis Reagent Pack
166-0460EDU	Large Fast Blast DNA Electrophoresis Reagent Pack
166-0451EDU	Small Ethidium Bromide DNA Electrophoresis Reagent Pack
166-0456EDU	Medium Ethidium Bromide DNA Electrophoresis Reagent Pack
166-0461EDU	Large Ethidium Bromide DNA Electrophoresis Reagent Pack
166-0477EDU	Gel Staining Trays, 4
166-0474EDU	Disposable Plastic Transfer Pipets, sterile, 500
166-0480EDU	Disposable Plastic Transfer Pipets, nonsterile, 500
166-0473EDU	Colored 1.5 ml Microcentrifuge Tubes, 6 colors, 600
223-9480EDU	1.5 ml EZ Micro™ Test Tubes, natural, 500
223-9430EDU	2 ml EZ Micro Test Tubes, natural, 500
166-0476EDU	Cell Culture Tubes, 17 x 100 mm, 14 ml, sterile, 25
TWI-0201EDU	0.2 ml Tubes With Domed Caps, natural, 1000
223-9500EDU	PCR Tube Capless Adaptors, 500
TRC-0501EDU	96-Place PCR-Tube Rack and Cover, 5

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