Microbial Growth Protocols

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Culture of *E. coli*

Materials required:

Reagents	Equipment
Pure *E. coli* culture	Cabinet incubator (37°C)
LB – Miller	Shaker incubator (37°C)
LB – Miller Plates	Culture dishes, sterile
	Culture flasks, sterile

Suspension culture of *E. coli*

Prepare LB medium by weighing appropriate powder medium and adding to water in a sterile flask

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Autoclave the broth and cool to room temperature

(Alternatively ready-to-use LB medium may be used)

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In a laminar flow chamber, transfer approximately 1 mL of overnight *E. coli* culture to the flask

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Seal the mouth of the flask with sterile cotton (non-absorbant) plugs; ensure the flask is not tightly sealed

↓

Incubate overnight at 37°C with continuous shaking

Plating *E. coli*

Prepare LB agar by weighing appropriate powder medium, agar and water to a sterile flask

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Autoclave the medium and cool just enough to be able to handle the flask

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In a laminar flow chamber, pour approximately 25-30 mL of the LB agar into sterile plates

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Allow the plates to set in the laminar flow chamber with lids slightly opened

(The plates may also be stored inverted at 4°C for future use)

(Alternatively, ready-to-use LB agar plates may be used)

↓	↓	↓
Lightly scratch the surface of frozen *E. coli* glycerol stock with a sterile inoculating loop	Pick up *E. coli* colony from a plate with culture with a sterile inoculating loop	Add 10-100 µL of *E. coli* suspension culture and add one the LB agar plate
↓	↓	↓
Streak the loop across the LB agar plate		Spread the culture all over the plate using a sterile glass spreader

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Invert and incubate the plates overnight at 37°C

Advanced Protocols

Plating Bacteriophage M13

Materials required:

Reagents	Equipment
Pure *E. coli* culture	Cabinet incubator (37°C)
LB agar EZMix™ (Product No. L7533)	Water Bath (47°C)
Appropriate selection antibiotic	Culture dishes
IPTG (Isopropyl-β-D-thiogalactoside) (Product No. I6758)	15 ml polypropylene culture tubes (sterile)
X-gal (Product No. B9146)	
Magnesium chloride	

Prepare a pure culture of *E. coli* by inoculating a single colony into 5 mL of LB or YT medium

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Prepare ten-fold serial dilutions of M13 bacteriophage stock in LB or YT medium

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Prepare LB agar or YT medium supplemented with 5mM $MgCl_2$in sterile test tubes; equilibrate at 47°C; add appropriate amounts of X-gal and IPTG solutions

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Add serially diluted M13 bacteriophage stocks 100 µL of pure bacterial culture; mix by gentle vortexing

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Pour the LB agar or YT medium with X-gal and IPTG to tubes containing infected bacteria; mix by gentle vortexing

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Transfer the contents to plate and swirl for even distribution of infected bacteria

↓

Allow the plates to set; invert and incubate the plates at 37°C

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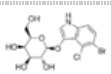
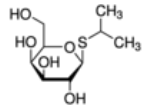
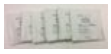
Pale blue plaques of M13 bacteriophage appear on a lawn of bacterial growth

The following are the bacterial broth components offered by Sigma-Aldrich:

Bacterial Broth Components	Name	Format
Y0875	**Select Yeast Extract**	Powder
N4767	**N-Z-Amine® EKC**	
Y1626	**Select Yeast Extract, EZMix™ powder**	EZMix™
T2559	**EZMix™ Tryptone**	

Materials

Product #	Image	Description	Molecular Formula	Add to Cart
Y2627		**2xYT Medium EZMix™ Powder microbial growth medium**		**pricing**
B9146		**5-Bromo-4-chloro-3-indolyl β-D-galactopyranoside ≥98%**	$C_{14}H_{15}BrClNO_6$	**pricing**
C4464		**EZMix™ N-Z-Amine® A Casein enzymatic hydrolysate, from bovine milk**		**pricing**
T2559		**EZMix™ Tryptone Pancreatic digest of casein**		**pricing**
H8032		**Hanahan's Broth (SOB Medium) powder microbial growth medium**		**pricing**
I6758		**IPTG ≥99% (TLC), ≤0.1% Dioxane**	$C_9H_{18}O_5S$	**pricing**
L3022		**LB Broth (Lennox) Highly-referenced microbial growth powder medium, low salt, suitable for salt-sensitive *E.coli* culture.**		**pricing**
L7275		**LB Broth (Lennox) Tablet microbial growth medium**		**pricing**

Product #	Image	Description	Molecular Formula	Add to Cart
L7658		LB Broth (Lennox) EZMix™ powder microbial growth medium		pricing
L3397		LB Broth (Luria low salt) Powder microbial growth medium		pricing
L3522		LB Broth (Miller) Highly-referenced nutrient-rich microbial growth powder medium, suitable for regular *E.coli* culture		pricing
L2542		LB Broth (Miller) Liquid microbial growth medium		pricing
L2897		LB Broth with agar (Lennox) Highly-referenced microbial growth powder medium with Agar, low salt, suitable for salt-sensitive E. coli culture.		pricing
L7025		LB Broth with agar (Lennox) Tablet microbial growth medium		pricing
L7533		LB Broth with agar (Lennox) EZMix™ powder microbial growth medium		pricing
L3272		LB Broth with agar (Luria low salt) Powder microbial growth medium		pricing
L3147		LB Broth with agar (Miller) Highly-referenced nutrient-rich microbial growth powder medium with Agar, suitable for regular *E.coli* culture.		pricing
M6030		M9, Minimal Salts, 5X powder, minimal microbial growth medium		pricing
N3518		NZ Amine® Broth Lambda bacteriophage growth medium		pricing
N4767		N-Z-Amine® EKC Casein enzymatic hydrolysate, from bovine milk		pricing
N3643		NZCYM Broth Powder microbial growth medium		pricing
N6905		NZCYM Broth EZMix™ powder microbial growth medium		pricing
Y0875		Select Yeast Extract for use in microbial growth medium		pricing
Y1626		Select Yeast Extract, EZMix™ powder for use in microbial growth medium		pricing
S1797		SOC Medium For use in transformation		pricing
T9179		Terrific Broth EZMix™ powder microbial growth medium		pricing
T5574		Terrific Broth Liquid microbial growth medium		pricing
Y2377		2x YT medium Powder microbial growth medium		pricing

References

- Sambrook, J., Fritsch, E.F., and Maniatis, T., Molecular cloning: a laboratory manual. New York: Cold spring harbor laboratory press, 1989.
- Parija, SC., Textbook of Microbiology and Immunology. Elsevier India, 2009.
- **http://www.scienceprofonline.org/microbiology/types-culture-media-for-growing-bacteria.html**