COMPREHENSIVE MINIMAL MEDIA PROTOCOL

Flexible Carbon Source System for Bacterial Growth Studies

Version 1.0
Application: Bacterial fitness testing in mealworm gut colonization studies
Primary Use: Testing inulin and other carbon sources with selective markers

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PROTOCOL OVERVIEW

Design Philosophy

This protocol separates the **base minimal media** (salts, buffers, trace elements) from **carbon sources** and **selective supplements**, allowing:

- Single batch of base media → multiple carbon source experiments
- Easy switching between test conditions
- Reduced waste and preparation time
- Consistent base composition across experiments

Workflow Summary

1. **Prepare base M9 minimal media** (autoclavable, no carbon)
2. **Prepare carbon source stocks separately** (filter sterilize)
3. **Prepare supplement stocks** (filter sterilize)
4. **Mix components aseptically** when ready to use

BASE MINIMAL MEDIA RECIPE

M9 Minimal Media (5X Stock - No Carbon Source)

M9 is the standard minimal media for *E. coli* and related bacteria. We'll prepare a **5X concentrated stock** of the base salts, then dilute to working concentration when making media.

5X M9 Salts Stock Solution (1 Liter)

Prepare this concentrated stock for long-term storage:

Component	Amount (per 1 L)	Final Concentration (5X)
Na_2HPO_4 (anhydrous)	33.9 g	64 g/L (5X)
KH_2PO_4	15.0 g	15 g/L (5X)
NaCl	2.5 g	2.5 g/L (5X)
NH_4Cl	5.0 g	5.0 g/L (5X)
Distilled water	to 1.0 L	-

Preparation:

1. Add components to ~800 mL distilled water
2. Stir until completely dissolved
3. Adjust to 1.0 L with distilled water
4. **Autoclave at 121°C for 20 minutes**
5. Store at room temperature (stable for 6+ months)

Note: If using $Na_2HPO_4 \cdot 7H_2O$ (heptahydrate), use 64.0 g instead of 33.9 g

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Essential Supplements (Prepare Separately)

These are added AFTER autoclaving the base media:

1. $MgSO_4$ Stock (1 M)

- **Preparation:** 24.6 g $MgSO_4 \cdot 7H_2O$ per 100 mL water
- **Sterilization:** Autoclave
- **Storage:** Room temperature, indefinite
- **Working concentration:** 2 mM final

2. $CaCl_2$ Stock (1 M)

- **Preparation:** 14.7 g $CaCl_2 \cdot 2H_2O$ per 100 mL water
- **Sterilization:** Autoclave
- **Storage:** Room temperature, indefinite
- **Working concentration:** 0.1 mM final

3. Trace Elements Solution (1000X)

Optional but recommended for optimal growth:

Component	Amount (per 100 mL)
$FeCl_3 \cdot 6H_2O$	0.81 g
$ZnCl_2$	0.13 g
$CuCl_2 \cdot 2H_2O$	0.013 g
$CoCl_2 \cdot 6H_2O$	0.12 g
H_3BO_3	0.062 g
$MnCl_2 \cdot 4H_2O$	0.081 g
Distilled water	to 100 mL

Preparation:

1. Dissolve in water (add a few drops of concentrated HCl to help dissolve iron)
2. **Filter sterilize (0.22 µm) -** DO NOT autoclave (will precipitate)
3. Store at 4°C in dark bottle (stable 6 months)

CARBON SOURCE STOCK SOLUTIONS

Prepare these separately and filter sterilize. Add to autoclaved base media when ready to use.

Standard Concentrations for Stock Solutions

Carbon Source	Stock Concentration	Working Concentration	Sterilization Method
Glucose	40% (w/v) = 400 g/L	0.2-0.4% (2-4 g/L)	Filter (0.22 µm)
Inulin	10% (w/v) = 100 g/L	0.2-0.5% (2-5 g/L)	Filter (0.22 µm)
Xylan	5% (w/v) = 50 g/L	0.2-0.5% (2-5 g/L)	Filter (0.22 µm)
Glycerol	50% (v/v)	0.2-0.4% (2-4 mL/L)	Autoclave OK

Detailed Preparation Protocols

Glucose Stock (40% w/v = 400 g/L)

For 100 mL stock:

1. Weigh 40 g D-glucose
2. Dissolve in ~80 mL distilled water with gentle heating (50°C) and stirring
3. Cool to room temperature
4. Adjust to 100 mL with distilled water
5. **Filter sterilize through 0.22 µm filter**
6. Store at 4°C (stable 6 months)

For 500 mL stock:

- 200 g glucose in 500 mL final volume

Inulin Stock (10% w/v = 100 g/L)

For 100 mL stock:

1. Weigh 10 g inulin (chicory-derived recommended)
2. Add to ~80 mL distilled water
3. Heat to 60-70°C with stirring to dissolve (may take 30-60 minutes)

4. Cool to room temperature
5. Adjust to 100 mL with distilled water
6. **Filter sterilize through 0.22 µm filter**
    - Note: May require pre-filtering through 0.45 µm if solution is cloudy
7. Store at 4°C (stable 3 months)

Important: Inulin solubility varies by chain length. If using high DP (degree of polymerization) inulin, heating time may be longer.

Xylan Stock (5% w/v = 50 g/L)

For 100 mL stock:

1. Weigh 5 g xylan (beechwood or oat spelt xylan)
2. Add to ~80 mL distilled water
3. Heat to 80°C with vigorous stirring (xylan is difficult to dissolve)
4. Stir for 1-2 hours at elevated temperature
5. Cool to room temperature (some precipitation may occur)
6. Centrifuge at 10,000 × g for 10 minutes if cloudy
7. **Filter sterilize supernatant through 0.45 µm, then 0.22 µm**
8. Store at 4°C (stable 2 months)

Note: Lower stock concentration due to poor solubility

Glycerol Stock (50% v/v)

For 100 mL stock:

1. Mix 50 mL glycerol (100%, reagent grade) with 50 mL distilled water
2. **Can autoclave** or filter sterilize
3. Store at room temperature (indefinite stability)

BATCH PREPARATION TABLES

Complete Minimal Media Recipe (1X Working Concentration)

Component	Small Batch (100 mL)	Medium Batch (500 mL)	Large Batch (1 L)
5X M9 Salts	20 mL	100 mL	200 mL

Component	Small Batch (100 mL)	Medium Batch (500 mL)	Large Batch (1 L)
Sterile H_2O	76 mL	380 mL	760 mL
1 M $MgSO_4$	200 µL	1.0 mL	2.0 mL
1 M $CaCl_2$	10 µL	50 µL	100 µL
Trace Elements (1000X)	100 µL	500 µL	1.0 mL
Carbon source stock	*See below*	*See below*	*See below*
TOTAL BEFORE CARBON	96.3 mL	481.5 mL	963 mL

Carbon Source Addition Table

For 0.4% (4 g/L) final glucose concentration:

Batch Size	Volume of 40% Glucose Stock to Add
100 mL	1.0 mL
500 mL	5.0 mL
1000 mL	10.0 mL

For 0.5% (5 g/L) final inulin concentration:

Batch Size	Volume of 10% Inulin Stock to Add
100 mL	5.0 mL
500 mL	25.0 mL
1000 mL	50.0 mL

For 0.4% (4 g/L) final xylan concentration:

Batch Size	Volume of 5% Xylan Stock to Add
100 mL	8.0 mL
500 mL	40.0 mL
1000 mL	80.0 mL

Agar Addition Table (for solid media)

Standard agar concentration: 1.5% (15 g/L)

Batch Size	Agar Amount	Note
100 mL	1.5 g	Add before autoclaving
250 mL	3.75 g	Good for ~10 plates
500 mL	7.5 g	Good for ~20 plates
1000 mL	15.0 g	Good for ~40 plates

For 1.2% "soft agar" (overlays): Use 12 g/L instead of 15 g/L

LIQUID MEDIA PREPARATION

Protocol: Preparing M9 Minimal Media (Liquid) with Carbon Source

Example: 500 mL of M9 + 0.4% Glucose

Materials Needed:

- Autoclaved 5X M9 salts stock
- Autoclaved distilled water (or sterile H_2O)
- 1 M $MgSO_4$ (autoclaved)
- 1 M $CaCl_2$ (autoclaved)
- Trace elements solution (filter sterilized)
- 40% glucose stock (filter sterilized)
- Sterile culture flasks or bottles

Step-by-Step:

1. **In a sterile flask or bottle, combine:**
    - 100 mL of 5X M9 salts
    - 380 mL sterile distilled water
    - 1.0 mL of 1 M $MgSO_4$
    - 50 µL of 1 M $CaCl_2$
    - 500 µL of trace elements solution (1000X)
2. **Mix well by swirling**
3. **Add carbon source:**
    - 5.0 mL of 40% glucose stock
    - (This gives final volume of ~486.5 mL, which is fine for "500 mL")
4. **Mix thoroughly**
5. **Label clearly:**
    - "M9 + 0.4% Glucose"
    - Date prepared
    - Your initials
6. **Store at 4°C until use** (use within 2 weeks)

For Multiple Carbon Sources from Same Base:

If you want to test multiple carbon sources, prepare the **base media without carbon**:

1. **Prepare "Complete M9 Base" (no carbon):**
    - For 400 mL base: 80 mL 5X M9 salts + 304 mL H_2O + 800 µL $MgSO_4$ + 40 µL $CaCl_2$ + 400 µL trace elements
2. **Aliquot into separate flasks:**
    - Flask 1: 95 mL base + 5 mL glucose stock → "M9 + Glucose"
    - Flask 2: 95 mL base + 5 mL inulin stock → "M9 + Inulin"
    - Flask 3: 95 mL base + 5 mL xylan stock → "M9 + Xylan"
    - Flask 4: 100 mL base + 0 mL carbon → "M9 No Carbon" (negative control)

AGAR PLATE PREPARATION

Protocol: Pouring M9 Minimal Media Agar Plates with Carbon Source

Example: 500 mL batch → approximately 20 plates (25 mL per plate)

Materials Needed:

- All components for liquid M9 media
- Agar (bacteriological grade)
- Autoclave-safe bottle (1 L capacity for 500 mL media)
- Water bath (55°C) or heating block
- Sterile petri dishes (100 × 15 mm)

Step-by-Step Protocol:

PART A: Prepare Base Agar (Before autoclaving)

1. **In a 1 L bottle, combine:**
    - 100 mL of 5X M9 salts
    - 380 mL distilled water
    - 7.5 g agar
    - Cap loosely (for autoclaving)
2. **Mix by swirling** (agar won't dissolve until autoclaving)
3. **Autoclave at 121°C for 20 minutes**
    - Liquid cycle
    - Allow pressure to return to zero before opening
4. **Cool in 55°C water bath for 30-45 minutes**
    - Media should be cool enough to handle but not solidified
    - Keep bottle caps loose to prevent pressure buildup during cooling

PART B: Add Supplements (After autoclaving)

5. **Once cooled to 55°C, add aseptically:**
    - 1.0 mL of 1 M $MgSO_4$
    - 50 µL of 1 M $CaCl_2$
    - 500 µL of trace elements solution
    - **5.0 mL of 40% glucose stock** (or your carbon source of choice)

6. **Add selective supplements if needed** (see Supplement Addition Guide)
7. **Mix gently** by swirling (avoid bubbles)

PART C: Pour Plates

8. **Work near flame** or in laminar flow hood
9. **Pour approximately 25 mL per plate:**
    - Open petri dish lid just enough to pour
    - Pour steadily until bottom is covered (~4-5 mm depth)
    - Replace lid immediately
    - 500 mL batch = ~20 plates
10. **Remove bubbles:**
    - Quickly pass flame over surface of liquid (don't melt plastic!)
    - Or use pipette tip to pop bubbles
11. **Let solidify:**
    - Leave plates undisturbed on level surface for 30-60 minutes
    - Lids should stay on during solidification
12. **Dry plates:**
    - Once solid, invert plates (lid down, agar up)
    - Leave overnight at room temperature OR
    - Place in 37°C incubator (lid-side down) for 1-2 hours with lids slightly ajar
13. **Storage:**
    - Once dry, seal plates in plastic bags
    - Store inverted at 4°C
    - Use within 2-3 weeks

Tips for Success:

- Don't add heat-sensitive supplements (5-FOA, antibiotics) until media is cooled to 50-55°C
- Work quickly but carefully when pouring to maintain sterility
- Keep bottle at 55°C (water bath) while pouring to prevent premature solidification
- If media starts to solidify while pouring, reheat gently in microwave (10-second bursts)

SUPPLEMENT ADDITION GUIDE

Heat-Sensitive Additions (Add After Cooling to 50-55°C)

5-FOA (5-Fluoroorotic Acid) - for pyrF Selection

Stock Solution: 10 mg/mL (10X)

Preparation:

1. Weigh 1.0 g 5-FOA powder
2. Dissolve in 100 mL of distilled water
    - May require heating to 50°C and stirring
    - Solution should be clear
3. **Filter sterilize through 0.22 µm filter**
4. Aliquot into 5-10 mL portions
5. Store at -20°C (stable for 6 months)

Working concentration: 1 mg/mL (1000 µg/mL) final

Addition amounts:

Media Volume	Volume of 10 mg/mL Stock to Add	Final Concentration
100 mL	10 mL	1 mg/mL
250 mL	25 mL	1 mg/mL
500 mL	50 mL	1 mg/mL
1000 mL	100 mL	1 mg/mL

Note: Add 5-FOA LAST, after all other supplements, when media has cooled to 50-55°C

Uracil - Supplement for pyrF Mutants

Stock Solution: 5 mg/mL (100X)

Preparation:

1. Weigh 0.5 g uracil
2. Add to ~80 mL distilled water

3. Heat to 60°C with stirring (uracil is poorly soluble at room temp)
4. Cool to room temperature
5. Adjust to 100 mL
6. **Filter sterilize through 0.22 µm filter**
7. Store at 4°C (stable 3 months)

Working concentration: 50 µg/mL final

Addition amounts:

Media Volume	Volume of 5 mg/mL Stock to Add	Final Concentration
100 mL	1.0 mL	50 µg/mL
500 mL	5.0 mL	50 µg/mL
1000 mL	10.0 mL	50 µg/mL

Antibiotic Stocks

Kanamycin Stock: 50 mg/mL (1000X)

- Dissolve 5 g kanamycin sulfate in 100 mL distilled water
- Filter sterilize (0.22 µm)
- Store at -20°C in 1-2 mL aliquots
- Working concentration: 50 µg/mL (add 1 mL per 1 L media)

Ampicillin Stock: 100 mg/mL (1000X)

- Dissolve 10 g ampicillin sodium salt in 100 mL distilled water
- Filter sterilize (0.22 µm)
- Store at -20°C in 1-2 mL aliquots
- Working concentration: 100 µg/mL (add 1 mL per 1 L media)
- **Note:** Ampicillin is less stable; make fresh aliquots monthly

Chloramphenicol Stock: 34 mg/mL (1000X)

- Dissolve 3.4 g chloramphenicol in 100 mL **100% ethanol**
- No need to filter sterilize (ethanol is sterile)

- Store at -20°C
- Working concentration: 34 μg/mL (add 1 mL per 1 L media)

Order of Addition (Critical!)

When preparing plates with multiple supplements, add in this order (from most stable to least stable):

1. **First:** $MgSO_4$, $CaCl_2$, Trace elements (heat stable)
2. **Second:** Carbon source (glucose, inulin, etc.)
3. **Third:** Uracil (if needed)
4. **Fourth:** Antibiotics (if needed)
5. **LAST:** 5-FOA (most heat-sensitive)

Wait until media cools to 50-55°C before adding anything from step 3 onward

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Quick Reference: Common Formulations

M9 + Inulin + 5-FOA Plates (for selective recovery)

For 500 mL (20 plates):

- 100 mL 5X M9 salts + 7.5 g agar + 380 mL H_2O → Autoclave
- Cool to 55°C, then add:
  - 1.0 mL $MgSO_4$
  - 50 μL $CaCl_2$
  - 500 μL trace elements
  - 25 mL inulin stock (10%)
  - 50 mL 5-FOA stock (10 mg/mL)
- Pour plates immediately

M9 + Glucose + Uracil (for pyrF mutant growth without selection)

For 500 mL:

- Base media (as above)
- After autoclaving and cooling:
  - 1.0 mL $MgSO_4$
  - 50 μL $CaCl_2$

- 500 µL trace elements
- 5 mL glucose stock (40%)
- 5 mL uracil stock (5 mg/mL)

STORAGE AND STABILITY

Stock Solutions

Solution	Storage Temp	Stability	Notes
5X M9 Salts	Room temp	6+ months	Watch for precipitation
$MgSO_4$ (1 M)	Room temp	Indefinite	-
$CaCl_2$ (1 M)	Room temp	Indefinite	-
Trace Elements	4°C, dark	6 months	Store in amber bottle
Glucose (40%)	4°C	6 months	Watch for contamination
Inulin (10%)	4°C	3 months	May precipitate over time
Xylan (5%)	4°C	2 months	Precipitates easily
Glycerol (50%)	Room temp	Indefinite	Very stable
5-FOA (10 mg/mL)	-20°C	6 months	Freeze-thaw stable
Uracil (5 mg/mL)	4°C	3 months	May crystallize if cold
Antibiotics	-20°C	Varies	See specific notes above

Prepared Media

Media Type	Storage	Shelf Life	Notes
Liquid M9 + Carbon	4°C	2 weeks	Use ASAP for best growth
M9 Agar Plates	4°C, sealed, inverted	2-3 weeks	Watch for drying, contamination
M9 + 5-FOA Plates	4°C, sealed, dark	2 weeks	5-FOA degrades slowly in light

Signs Media Needs to Be Replaced:

Liquid Media:

- Cloudiness (contamination)
- Color change
- pH shift (if using pH indicator)
- Poor growth in positive controls

Agar Plates:

- Visible contamination (colonies appearing)
- Excessive drying (plates look shrunken)
- Discoloration
- Poor growth of positive controls

QUALITY CONTROL

Testing New Media Batches

Always validate new batches before using in critical experiments:

Positive Control Test

Purpose: Verify media supports growth

Protocol:

1. Inoculate fresh plate or broth with your bacterial strain
2. Use a strain you KNOW grows well on this media
3. Incubate under standard conditions
4. Expected result: Robust growth within 18-24 hours (for fast-growing organisms like *E. coli*)

Acceptance criteria:

- Colony size normal
- Growth rate comparable to previous batches
- Colony morphology normal

Negative Control Test

Purpose: Verify sterility

Protocol:

1. Incubate uninoculated plate or broth
2. Same incubation conditions as experimental samples
3. Expected result: No growth

Acceptance criteria:

- No visible colonies or turbidity after 48-72 hours

Selection Marker Test (for 5-FOA plates)

Purpose: Verify selective pressure works

Protocol:

1. Plate pyrF+ strain (wild-type) on M9 + 5-FOA plate
2. Plate pyrF- strain (mutant) on same media
3. Incubate 24-48 hours
4. Expected results:
    - pyrF+ (wild-type): No growth or very minimal growth
    - pyrF- (mutant): Normal growth

Acceptance criteria:

- Wild-type shows >99% killing
- Mutant grows normally

Carbon Source Utilization Test

Purpose: Verify carbon source is available to bacteria

Protocol:

1. Streak same strain on:
    - M9 + glucose (positive control)
    - M9 + inulin (test)
    - M9 no carbon (negative control)
2. Incubate 24-48 hours
3. Expected results:

- Glucose: Good growth
- Inulin: Growth only if strain has inulinase genes
- No carbon: No growth or minimal growth

Troubleshooting Common Issues

Problem: Plates dry out quickly

Possible causes:

- Plates not sealed properly
- Incubator too dry
- Agar concentration too low

Solutions:

- Seal plates in plastic bags after drying
- Add water tray to incubator
- Increase agar to 1.8%

Problem: Precipitation in media

Possible causes:

- pH too high or too low
- Incompatible salts (especially Ca^{2+} and phosphate)
- Overheating trace elements

Solutions:

- Check pH of 5X M9 stock (should be ~7.4)
- Always add $CaCl_2$ last and in diluted form
- Never autoclave trace elements solution
- If persistent, prepare fresh stocks

Problem: Poor growth on minimal media

Possible causes:

- Missing essential supplements
- Carbon source not utilized by strain
- Incorrect pH
- Old media components

Solutions:

- Verify strain genotype (auxotrophies)
- Test on M9 + glucose first (known to work)
- Check pH (should be 7.0-7.4)
- Prepare fresh stocks, especially trace elements
- Consider adding amino acids for auxotrophs

Problem: 5-FOA selection not working

Possible causes:

- 5-FOA degraded (old stock or light exposure)
- Concentration too low
- pH incorrect
- Residual uracil in media

Solutions:

- Use fresh 5-FOA stock (<6 months old)
- Store in dark at -20°C
- Verify final concentration (1 mg/mL)
- Check pH (5-FOA works best at pH 6.5-7.5)
- Do not add uracil to 5-FOA selection plates!

Problem: Inulin won't dissolve

Possible causes:

- High DP (degree of polymerization) inulin

- Temperature too low
- Concentration too high

Solutions:

- Heat to 70-80°C with vigorous stirring
- Extend heating time to 1-2 hours
- Reduce stock concentration to 5% w/v
- Source chicory inulin (more soluble than other types)
- Consider sonicating solution

QUICK REFERENCE CARDS

Card 1: Standard M9 Liquid Media Recipes

M9 + 0.4% Glucose (500 mL)

- 100 mL 5X M9 salts
- 380 mL sterile H_2O
- 1.0 mL $MgSO_4$ (1 M)
- 50 µL $CaCl_2$ (1 M)
- 500 µL trace elements
- 5 mL glucose stock (40%)

M9 + 0.5% Inulin (500 mL)

- 100 mL 5X M9 salts
- 380 mL sterile H_2O
- 1.0 mL $MgSO_4$ (1 M)
- 50 µL $CaCl_2$ (1 M)
- 500 µL trace elements
- 25 mL inulin stock (10%)

Card 2: Standard Agar Plate Recipes

M9 + 0.4% Glucose Plates (500 mL = 20 plates) *Before autoclaving:*

- 100 mL 5X M9 salts
- 380 mL H_2O
- 7.5 g agar

After autoclaving, cool to 55°C, add:

- 1.0 mL $MgSO_4$
- 50 µL $CaCl_2$
- 500 µL trace elements
- 5 mL glucose stock

M9 + 0.5% Inulin + 5-FOA Plates (500 mL = 20 plates) *Before autoclaving:*

- 100 mL 5X M9 salts
- 380 mL H_2O
- 7.5 g agar

After autoclaving, cool to 55°C, add:

- 1.0 mL $MgSO_4$
- 50 µL $CaCl_2$
- 500 µL trace elements
- 25 mL inulin stock
- 50 mL 5-FOA stock (ADD LAST!)

Card 3: Quick Conversion Table

Carbon Source Stock Concentrations:

- Glucose: 40% (400 g/L)
- Inulin: 10% (100 g/L)
- Xylan: 5% (50 g/L)
- Glycerol: 50% (v/v)

For 0.4% final concentration in different volumes:

Volume	Glucose (40%)	Inulin (10%)*	Xylan (5%)**
100 mL	1 mL	4 mL	8 mL
250 mL	2.5 mL	10 mL	20 mL
500 mL	5 mL	20 mL	40 mL
1000 mL	10 mL	40 mL	80 mL

*For 0.4% inulin from 10% stock
**For 0.4% xylan from 5% stock

Card 4: Supplement Concentrations

Supplement	Stock	Working	Add to 500 mL
$MgSO_4$	1 M	2 mM	1.0 mL
$CaCl_2$	1 M	0.1 mM	50 µL
Trace Elements	1000X	1X	500 µL
5-FOA	10 mg/mL	1 mg/mL	50 mL
Uracil	5 mg/mL	50 µg/mL	5 mL
Kanamycin	50 mg/mL	50 µg/mL	0.5 mL

EXPERIMENTAL DESIGN NOTES

For Your Inulin Selection Experiments:

Pre-Feeding Protocol:

Goal: Enrich bacteria with inulin metabolism capability

1. **Inoculate** strain into M9 + 0.5% inulin liquid media
2. **Grow** for 24-48 hours at optimal temperature (37°C for *E. coli*)
3. **Dilute** and feed to mealworms

4. **Monitor** CFU recovery

Selective Recovery Protocol:

Goal: Recover pyrF mutants that colonized gut using inulin

1. **Extract** mealworm guts
2. **Homogenize** in sterile buffer (PBS or M9 salts)
3. **Serially dilute** homogenate
4. **Plate** on M9 + 0.5% inulin + 5-FOA plates
5. **Incubate** 24-48 hours
6. **Count** CFU (only pyrF- mutants that can use inulin will grow)

Controls to Include:

- **Positive control:** M9 + glucose + 5-FOA (pyrF- should grow)
- **Negative control:** M9 + 5-FOA no carbon (no growth expected)
- **Wild-type control:** M9 + inulin + 5-FOA (wild-type should NOT grow)
- **Growth control:** M9 + inulin no 5-FOA (all strains that use inulin grow)

NOTES AND CALCULATIONS

Converting Between Carbon Source Concentrations

Formula: $C_1V_1 = C_2V_2$

Where:

- C_1 = stock concentration
- V_1 = volume of stock to add
- C_2 = desired final concentration
- V_2 = final total volume

Example: To get 0.5% (5 g/L) inulin in 500 mL final volume from 10% (100 g/L) stock:

- $(100\ g/L) \times V_1 = (5\ g/L) \times (500\ mL)$
- $V_1 = (5 \times 500) / 100 = 25\ mL$

Calculating Plate Numbers

Standard petri dish: 100 mm × 15 mm (diameter × depth)
Standard volume per plate: 20-25 mL
Typical yield: 500 mL media → 20-25 plates

If you need more plates:

- For 40 plates: Prepare 1 L media
- For 100 plates: Prepare 2.5 L media (make two separate batches)

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pH Considerations

M9 minimal media pH: Should be 7.0-7.4

The phosphate buffer in M9 maintains pH, but:

- **If pH too high (>7.6):** Nutrients may precipitate
- **If pH too low (<6.8):** Some bacteria grow poorly

To adjust pH:

- Lower pH: Add 1 M HCl dropwise
- Raise pH: Add 1 M NaOH dropwise
- Always check pH before autoclaving and after adding all supplements

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DOCUMENT REVISION HISTORY

Version	Date	Changes	Author
1.0	[Today]	Initial protocol creation	Chris

FINAL CHECKLIST

Before starting an experiment, verify:

- [ ] Fresh 5X M9 salts stock available
- [ ] Carbon source stocks prepared and sterile

- [ ] Supplement stocks prepared (5-FOA, uracil, etc.)
- [ ] $MgSO_4$ and $CaCl_2$ stocks available
- [ ] Trace elements solution fresh (<6 months old)
- [ ] Agar (if making plates)
- [ ] Sterile petri dishes
- [ ] All stocks stored at proper temperatures
- [ ] Positive and negative controls planned
- [ ] Lab notebook entry prepared with:
- [ ] Date and time
- [ ] Batch numbers of all stocks used
- [ ] Calculations checked
- [ ] Expected results defined

END OF PROTOCOL

For questions or troubleshooting, consult lab manager or refer to:

- *Sambrook & Russell, Molecular Cloning (Cold Spring Harbor Press)*
- *Miller, J.H. (1992) A Short Course in Bacterial Genetics (CSHL Press)*