

Presence/Absence

Methods 8319 and 8364

P/A Broth (8319)

P/A Broth w/ MUG (8364)

Scope and Application: For water and wastewater



Test preparation

Before starting the test:

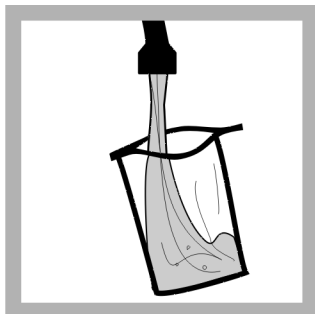
Disinfect the work bench with a germicidal cloth, dilute bleach solution, bactericidal spray, or dilute iodine solution

Wash hands thoroughly with soap and water.

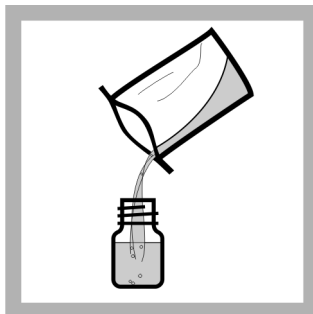
Introduction

Both P/A Broth and P/A Broth with MUG come packaged in disposable bottles and in glass ampules. The medium in both bottles and ampules is concentrated and must be diluted with sterile water 5:1. The medium is sterilized by membrane filtration to prevent degradation.

P/A Broth ampules, method 8319



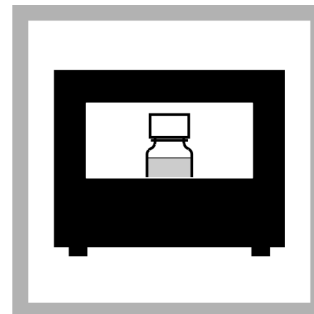
1. Collect 100 mL of sample in a sterile sample container. Use aseptic technique to avoid contaminating the sample and sample container.



2. Add sample to the fill-to line of a sample bottle. Sample may be added from a sterile container, or directly from a faucet or spigot.



3. Add the contents of one P/A Broth ampule to the 100 mL of sample.



4. Incubate the sample at 35 ± 0.5 °C for 24–48 hours.

P/A Broth ampules, method 8319



Confirm Positive Samples

Dispose of all completed tests

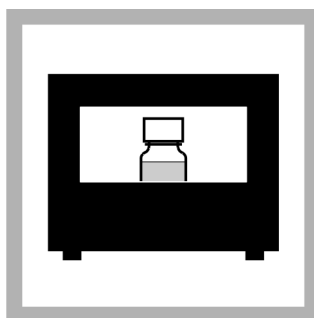
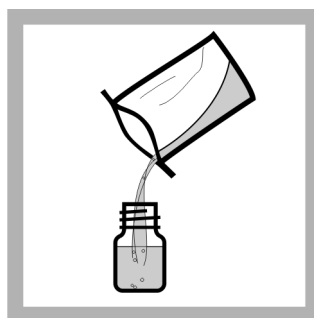
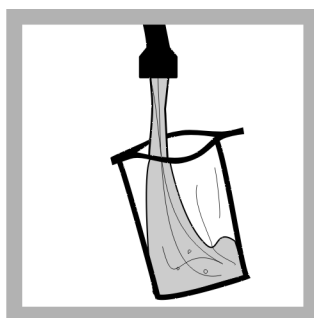
5. Note the reaction after 24 hours of incubation. See *Interpret and report results* on page 3.

6. Confirm presumptive positive samples by inoculating the appropriate media directly from positive P/A broth cultures. See the *Confirmation Table* on page 3.

7. Dispose of all completed tests appropriately.

Dispose of all completed tests

P/A broth with MUG, method 8364



1. Collect 100 mL of sample in a sterile sample container. Use aseptic technique to avoid contaminating the sample and sample container.

2. Add sample to the fill-to line of a Broth Disposable Bottle. Sample may be added from a sterile container, or directly from a faucet or spigot.

3. Incubate the sample at 35 ± 0.5 °C for 24–48 hours.

4. Note the reaction after 24 hours of incubation. See [Interpret and report results](#).

P/A broth with MUG, method 8364 (continued)

Confirm Positive Samples

Dispose of all completed tests

5. Confirm presumptive positive samples by inoculating the appropriate media directly from positive P/A broth cultures. See the [Confirmation Table](#).

6. Dispose of all completed tests appropriately.

Interpret and report results

Reactions using P/A Broth

- *Color change from reddish purple to yellow or yellow brown*—record the test as presumptive positive for total coliform bacteria.
- *No color change*—incubate for an additional 24 hours and recheck the sample for color change.

If after 24 hours of incubation, the *color changes from reddish purple to yellow or yellow brown*—record the test as presumptive positive for total coliform bacteria.

If after 48 ±3 hours of incubation, the sample *still appears reddish purple*—record the test as negative for total coliform bacteria.

Reactions using P/A Broth with MUG

- Examine the sample under long-wave UV light; *if the sample fluoresces*—record the test as positive for *E. coli*.

Confirm positive samples

Inoculum from incubated presence/absence samples are used to confirm the presence of total coliforms, fecal coliforms or *E. coli* in the original water sample. Use a sterile inoculating loop to transfer sample to an appropriate confirmation medium.

Table 1 Confirmation Table

Bacteria	Confirmation Medium	Incubation	Incubator	Positive Result
Total Coliform	Brilliant Green Bile Broth (322-15)	24 to 48 hours. 35 ± 0.5 °C	Culture (2619200 or -02) or 12-well Dri-Bath (2281400)	Gas/Turbidity
Fecal Coliform	EC Medium Tubes (14104-15)	24 hours 44.5 ± 0.2 °C	12-well Dri-Bath (2281400)	Gas/Turbidity
<i>E. coli</i>	EC Medium with MUG Tubes (24715-15)	24 hours 44.5 ± 0.2 °C	12-well Dri-Bath (2281400)	Fluorescence

Controls

Positive and negative controls are important. *Pseudomonas aeruginosa* is recommended as a negative control and *Escherichia coli* as a positive control. Use the AQUA QC-STIK™ Device for quality control procedures. Instructions for use come with each AQUA QC-STIK Device. Potable water samples from municipal treatment facilities should be negative for total coliforms and fecal coliforms.

Completed test disposal

Active bacterial cultures grown during incubation must be disposed of safely. Use either [Bleach](#) or an [Autoclave](#) to neutralize bacteria.

Bleach

1. Sterilize used test containers with a 10% bleach solution.
2. Add approximately 12 mL of bleach to each test container and allow to stand for 10 to 15 minutes.
3. Pour the liquid down the drain. Dispose of the test tubes with the normal garbage.

Autoclave

Test containers must be placed in a bag before autoclaving to prevent leakage of solution into the autoclave.

1. Place used test tubes in a contaminated-items bag or a biohazard bag and seal tightly.
1. Autoclave the used test tubes in the bag at 121 °C for 15 minutes at 15 pounds of pressure.
2. Place the bag of test tubes in a separate garbage bag and tie tightly.
3. Once the test tubes are sterile, dispose of them with the normal garbage.

Summary of method 8319

P/A Broth is ideal for screening drinking water samples for total coliforms. The method is a simple modification of the multiple-tube method. It uses lactose and lauryl tryptose broths with bromcresol purple, which detects acidity formed during lactose fermentation by the bacteria.

Simply combine 100 mL of sample and P/A Broth, incubate for 24 hours and check for a color change. A yellow color indicates the presence of total coliforms.

Summary of method 8364

P/A Broth with MUG allows simultaneous detection of total coliform bacteria and *E. coli*. In addition to the lactose and lauryl tryptose broths with bromcresol purple, this medium contains MUG reagent (4-methylumbelliferyl-b-D-glucuronide). The MUG reagent produces a fluorogenic product when hydrolyzed by glucuronidase (an enzyme specific to *E. coli*). MUG detects non gas-producing (anaerogenic) strains of *E. coli* and works well when competitive organisms are present.

Combine 100 mL of sample and P/A Broth with MUG, incubate for 24 hours and check for a color change and fluorescence. A yellow color indicates the presence of total coliforms. To detect *E. coli*, examine samples under a long-wave ultraviolet (UV) light. Fluorescence indicates the presence of *E. coli*.

Consumables and replacement items

Required media and reagents

Description	Unit	Catalog number
P/A Broth Ampules		
P/A Broth Ampules	25/pkg	2494925
P/A Broth with MUG Ampules	25/pkg	2495525
P/A Broth in Disposable Bottles		
P/A Broth Disposable Bottles	12/pkg	2323212
P/A Broth Disposable Bottles	50/pkg	2323250
P/A Broth with MUG Disposable Bottles	12/pkg	2401612
P/A Broth with MUG Disposable Bottles	50/pkg	2401650

Required apparatus

Description	Unit	Catalog number
Bottles, sample, presterilized, 100-mL fill-to line	12/pkg	2495012
Incubator, Culture, low profile, 110/120 VAC, 50/60 Hz	each	2619200
Incubator, Culture, low profile, 220/240 VAC, 50/60 Hz	each	2619202
Lamp, handheld, long-wave, ultraviolet, 115 VAC, 60 Hz	each	2184300
Lamp, handheld, long-wave, ultraviolet, 230 VAC, 50 Hz	each	2184302

Optional apparatus

Description	Unit	Catalog number
Autoclave, 120 VAC, 50/60 Hz	each	2898600
Bags for contaminated items	200/pkg	2463300
Bags, Whirl-Pak [®] 1, with dechlorinating agent	100/pkg	2075333
Bottles, sample, presterilized, 100-mL fill-to line	50/pkg	2495050
Bottles, sample, presterilized, with dechlorinating agent, 100-mL fill-to line	12/pkg	2599112
Bottles, sample, presterilized, with dechlorinating agent, 100-mL fill-to line	12/pkg	2599150
Lamp, long-wave, ultraviolet, portable	each	2415200
Incubator, Dri-Bath, 12-Well, 120V	each	2281400
Bags, Whirl-Pak ¹ , without dechlor 207 mL	100/pkg	2233199
Bags, Whirl-Pak ¹ , without dechlor 720 mL	10/pkg	1437297
Dechlorinating reagent, powder pillows	100/pkg)	1436369
Inoculating loop, nichrome V wire	each	2112100
Inoculating loop, disposable	25/pkg	2749125
Isopropyl alcohol	500 mL	1445949
Alcohol burner	each	2087742
Replacement wicks for 2087742	each	2097810
Incubator, portable, 12 VDC	each	2569900
Battery eliminator	each	2580400

Coliforms

Optional apparatus

Description	Unit	Catalog number
Rechargeable battery pack, for portable incubator 12V DC / 115 V AC adapter	each	2580300
230V AC Rechargeable batter pack adapter (for 2580300)	rach	2595902

¹ Whirl-Pak is a registered trademark of Nasco, Inc.

Optional media

Description	Unit	Catalog number
Bottles, sample, presterilized, 100-mL fill-to line	12/pkg	2495012
Incubator, Culture, low profile, 110/120 VAC, 50/60 Hz	each	2619200
Incubator, Culture, low profile, 220/240 VAC, 50/60 Hz	each	2619202
Lamp, handheld, long-wave, ultraviolet, 115 VAC, 60 Hz	each	2184300
Lamp, handheld, long-wave, ultraviolet, 230 VAC, 50 Hz	each	2184302



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