

ALKEN-MURRAY CORPORATION	TITLE: PLATE COUNT (PCA) AGAR PREPARATION PROCEDURE	NO. QC-24
MICROBIOLOGICAL DIVISION	QUALITY CONTROL	REV: 3
	PAGE 1 OF 3	

# ALKEN-MURRAY CORPORATION

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## QUALITY CONTROL METHOD - 24

Preparation of Plate Count (PCA) Agar

### Description:

This quality control procedure is designed to verify that PCA agar, alternate agar used in QC-1, QC-3, is obtained and stored properly. This procedure should be performed by a trained laboratory technician.

### Equipment & Supplies:

- Incubator
- Sterilizer or autoclave
- Orbital incubator-shaker
- Chemical balance ( $\geq 200$  g capacity; 0.01 gram precision)
- Heated stir plate and magnetic stir bar
- pH meter with dual probe for temperature and pH
- Eppendorf* Adjustable Air Displacement Pipetter
- 1 Erlenmeyer flask or beaker (1000 ml - or double the volume of media being prepared)
- 2 Weighing dishes (not necessary to sterilize for media preparation)
- 1 500 ml glass measuring cylinder
- 1 10 ml syringe
- 1 Calibrated pH solution to verify pH meter reading
- 1 Sterile petri dish per sample tested
- 1 Sterile bacterial spreader per sample tested
- 1 pipet tip for each sample to be inoculated
- 1 Sterile Erlenmeyer flask (250 ml) for each sample that must be rehydrated for testing
- 1 Sterile weighing dish per sample to be tested
- 1 Wash bottle with 500 ml of distilled water
- 1 Steri-Wrap II (green) closure (cut to size) with rubber bands for security
- 1 Bellco silicone sponge or Rapid-Flo double-gauze milk filter disks (for closure strength)

### Ingredients:

- PCA (Weber #3097-11) 11.75 grams
- Distilled water 500 ml

ALKEN-MURRAY CORPORATION	TITLE: PLATE COUNT (PCA) AGAR PREPARATION PROCEDURE	NO. QC-24
MICROBIOLOGICAL DIVISION	QUALITY CONTROL	REV: 3
		PAGE 2 OF 3

## Procedure:

1. Purchase PCA Agar from Weber Scientific.
2. Place a label on package with expiration date marked in bold RED letters
3. Store powdered agar below 30°C
4. To prepare agar, dissolve 11.75 grams of powder agar in 500 ml distilled water
5. Insert stirbar in flask and apply closure to the flask.
  - 5.1 A 250 ml flask closure consists of a Bellco silicone sponge stopper (or equivalent) covered with double layer of green steri-wrap II (or equivalent) and secured with rubber bands.
  - 5.2 A 2 liter flask closure consists of a double layer of steri-wrap (or equivalent) with a double-gauze milk filter (or equivalent) sandwiched in between secured with rubber bands
6. Heat with frequent agitation to boil. Boil for 1 minute to completely dissolve the powder.
7. Autoclave for 15 minutes at 121°C before use.
8. Once media has cooled to 45°C, dispense 15-20 ml of PCA agar per sterile, plastic disposable petri dish (enough to just coat the bottom of the dish).
9. Store prepared agar in the refrigerator, in the dark, until used. Use prepared agar within one week.
10. Prepare bacteria according to instructions in QC-1 or the QC procedure that calls for this agar.
11. Add 2.0 grams of dry product to the sterile phosphate buffer solution in the buffer bottle. Shake by hand to mix. Place the bottle on an orbital shaker for 30 minutes at 150 cycles per minute at 35°C.
12. Place one sterile gauze sponge in the funnel, supported on a ring stand, above an Erlenmeyer flask. Pour the buffer bottle contents into the gauze pad, to filter it. Use the wash bottle to direct a fine stream of water into the buffer bottle, to rinse out all the remaining bran in the gauze pad. Use as little water as possible to do this. Then partly fill the bottle with distilled water, shake it and discard the rinse water.

ALKEN-MURRAY CORPORATION	TITLE: PLATE COUNT (PCA) AGAR PREPARATION PROCEDURE	NO. QC-24
MICROBIOLOGICAL DIVISION	QUALITY CONTROL	REV: 3
		PAGE 3 OF 3

13. With sterile gloves on your hands, squeeze the gauze pad, to gather any further bacterial culture into the collection flask. Return the filtrate to the bottle.
14. When testing liquid products, dilute with 20% distilled water or phosphate buffer and shake in incubator as mentioned in item 14. above, but filtration is not necessary.
15. Use *Eppendorf* pipetter to dispense 0.1 ml onto the surface of each petri dish. See QC-1 for techniques to use air displacement pipetter.
16. Use sterile cell spreader to lightly and evenly spread bacteria thinly around the petri dish.
17. Incubate plates inverted at 32° to 35°C (90° to 95°F) for 24 hours.