

ALKEN-MURRAY CORPORATION	TITLE: TRYPTICASE SOY BROTH/AGAR PREPARATION PROCEDURE	NO. QC-22
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# ALKEN-MURRAY CORPORATION

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## QUALITY CONTROL METHOD - 22

Preparation of Trypticase Soy Broth (ATCC Culture Medium 18)

### Description:

This quality control procedure is designed to reproducibly prepare trypticase soy broth flasks for use in various quality control and fermentation procedures. Trypticase soy broth is a basic medium used for culturing many kinds of microorganisms. Trypticase soy agar is mainly used as an initial growth medium for the following purposes:

- ◆ observe colony morphology
- ◆ develop a pure culture
- ◆ achieve sufficient growth for further biochemical testing
- ◆ culture storage

Trypticase soy broth is used mostly to generate a large supply of bacteria for certain biochemical tests. It can also be used in the determination of bacterial numbers. This procedure should be performed by a trained laboratory technician.

### Equipment:

3 - 2 liter Erlenmeyer flasks or 6 250 ml flasks  
Bellco silicone sponge stoppers and/or Rapid-Flo double-gauze milk filter disks  
Balance sensitive to 0.001 g  
green Steri-Wrap II  
rubber bands  
autoclave

### Ingredients:

Trypticase Soy broth (Weber #3089-00)	30g
or	
(Weber #3089-04)	30g
Agar (Difco # 0140-01-0)	15g
Deionized, distilled water	1.0 L

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**Procedure:**

1. Purchase Trypticase Soy broth base from Weber Scientific.
2. Place a label on package with expiration date marked in bold RED letters
3. Store powder between 2° and 30°C
4. Add 30g broth base to 1 liter of deionized water or a multiple of the same ratio.
5. Dispense approximately 100 mls into 250ml Erlenmeyer Flasks or approximately 500 mls into 1 liter Erlenmeyer Flasks using the flask volume graduations.
6. Apply closure to each flask.
  - 6.1 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.
  - 6.2 A 1 - 2 liter flask closure consists of a double layer of Steri-Wrap II (or equivalent) with a single gauze milk filter (or equivalent) sandwiched in between secured with rubber bands.
7. Warm gently to until media is totally dissolved.
8. Sterilize in autoclave for 15 - 20 minutes at 121°C
9. Allow flask to cool to room temperature..
10. Store prepared broth in the refrigerator, in the dark, until used.
11. Adjust temperature to 30°C for culturing *Corynebacterium sp 995*
12. Adjust temperature to 37°C for culturing *Bacillus Megaterium de Bary 300*
13. Adjust temperature to 40°C for culturing *Aquaspirillum arcticum 402*

For Agar:

1. Prepare according to procedure QC-11, using 40 grams of medium to 1 liter of distilled water.
2. Heat and stir to boiling. Boil for 1 minute to dissolve completely.
3. Autoclave for 15 minutes at 121°C (15 lbs pressure) and cool to 45°C before dispensing into petri dishes.