

§ 436.102

21 CFR Ch. I (4-1-98 Edition)

Methyl alcohol: 50.0 ml.  
1 percent potassium phosphate buffer, pH 6.0,  
q.s.: 1,000.0 ml.

(18) *Solution 18 (0.054M sodium phosphate buffer, pH 6.9).*

Sodium dihydrogen phosphate monohydrate: 3.97 gm.  
Disodium hydrogen phosphate anhydrous: 3.55 gm.  
Distilled water, q.s.: 1,000.0 mL.

[39 FR 18944, May 30, 1974, as amended at 40 FR 52004, Nov. 7, 1975; 45 FR 75194, Nov. 14, 1980; 47 FR 9396, Mar. 5, 1982]

§ 436.102 Culture media.

(a) *Ingredients.* Use ingredients that conform to the standards, if any, prescribed by the U.S.P. or N.F. In lieu of preparing the media from the individual ingredients specified, they may be made from dehydrated mixtures that, when reconstituted with distilled water, have the same composition as such media. Minor modifications of the individual ingredients specified in this section are permissible if the resulting media possess growth-promoting properties at least equal to the media described.

(b) *Description of media.* Medium numbers 1, 2, 3, 4, 5, 8, 9, 10, 11, and 13 correspond to those used in "Assay Methods of Antibiotics," D. C. Grove and W. A. Randall, Medical Encyclopedia, Inc., New York, N.Y. (1955) p. 220, which is incorporated by reference. Copies are available from Medical Encyclopedia Inc., 30 East 60th St., New York, NY, or available for inspection at the Office of the Federal Register, 800 North Capitol Street, NW., suite 700, Washington, DC. Medium numbers 18 through 21 correspond to those used in "Outline of Details for Official Microbiological Assays of Antibiotics," A. Kirshbaum and B. Arret, "Journal of Pharmaceutical Sciences," vol. 56, No. 4, April 1967, p. 512, which is incorporated by reference. Copies are available from the American Pharmaceutical Association, 2215 Constitution Ave. NW., Washington, DC 20037, or available for inspection at the Office of the Federal Register (see address in this paragraph).

(1) *Medium 1.*

Peptone: 6.0 gm.  
Pancreatic digest of casein: 4.0 gm.  
Yeast extract: 3.0 gm.  
Beef extract: 1.5 gm.

Dextrose: 1.0 gm.  
Agar: 15.0 gm.  
Distilled water, q.s: 1,000.0 ml.  
pH 6.5 to 6.6 after sterilization.

(2) *Medium 2.*

Peptone: 6.0 gm.  
Yeast extract: 3.0 gm.  
Beef extract: 1.5 gm.  
Agar: 15.0 gm.  
Distilled water, q.s: 1,000.0 ml.  
pH 6.5 to 6.6 after sterilization.

(3) *Medium 3.*

Peptone: 5.0 gm.  
Yeast extract: 1.5 gm.  
Beef extract: 1.5 gm.  
Sodium chloride: 3.5 gm.  
Dextrose: 1.0 gm.  
Dipotassium phosphate: 3.68 gm.  
Potassium dihydrogen phosphate: 1.32 gm.  
Distilled water, q.s: 1,000.0 ml.  
pH 6.95 to 7.05 after sterilization.

(4) *Medium 4.*

Peptone: 6.0 gm.  
Yeast extract: 3.0 gm.  
Beef extract: 1.5 gm.  
Dextrose: 1.0 gm.  
Agar: 15.0 gm.  
Distilled water, q.s: 1,000.0 ml.  
pH 6.5 to 6.6 after sterilization.

(5) *Medium 5.* Medium 5 is the same as medium 2, except adjust the final pH to 7.8 to 8.0 after sterilization.

(6)-(7) [Reserved]

(8) *Medium 8.* Medium 8 is the same as medium 2, except adjust the final pH to 5.8 to 6.0 after sterilization.

(9) *Medium 9.*

Pancreatic digest of casein: 17.0 gm.  
Papaic digest of soybean: 3.0 gm.  
Sodium chloride: 5.0 gm.  
Dipotassium phosphate: 2.5 gm.  
Dextrose: 2.5 gm.  
Agar: 20.0 gm.  
Distilled water, q.s: 1,000.0 ml.  
pH 7.2 to 7.3 after sterilization.

(10) *Medium 10.* Medium 10 is the same as medium 9, except:

Agar: 12.0 gm.  
Polysorbate 80 (add polysorbate 80 after boiling the medium to dissolve the agar): 10.0 ml.  
pH 7.2 to 7.3 after sterilization.

(11) *Medium 11.* Medium 11 is the same as medium 1, except adjust the final pH to 7.8 to 8.0 after sterilization.

(12) [Reserved]

(13) *Medium 13.*

Peptone: 10.0 gm.

Dextrose: 20.0 gm.  
Distilled water, q.s.: 1,000.0 ml.  
pH 5.6 to 5.7 after sterilization.

(14)–(18) [Reserved]  
(19) *Medium 19.*

Peptone: 9.4 gm.  
Yeast extract: 4.7 gm.  
Beef extract: 2.4 gm.  
Sodium chloride: 10.0 gm.  
Dextrose: 10.0 gm.  
Agar: 23.5 gm.  
Distilled water, q.s.: 1,000.0 ml.  
pH 6.0 to 6.2 after sterilization.

(20)–(31) [Reserved]

(32) *Medium 32.* Prepare as medium 1, except add 300 milligrams of hydrated manganese sulfate (MnSO<sub>4</sub>·H<sub>2</sub>O) to each liter of medium.

(33) *Medium 33.* Use medium 1, sterilized and cooled to 50° C. Aseptically add sufficient sterile sodium novobiocin solution to give a final concentration of 10 micrograms of novobiocin activity per milliliter of medium. Sterile sodium novobiocin solution is prepared by filtering a solution containing 2.5 milligrams of novobiocin per milliliter of distilled water through a membrane filter of 0.22-micron porosity.

(34) *Medium 34.*

Glycerol: 10.0 gm.  
Peptone: 10.0 gm.  
Beef extract: 10.0 gm.  
Sodium chloride: 3.0 gm.  
Distilled water, q.s.: 1,000.0 ml.  
pH 7.0 after sterilization.

(35) *Medium 35.* Same as medium 34, except add 17.0 grams of agar to each liter of medium.

(36) *Medium 36.*

Pancreatic digest of casein ..... 15.0 gm.  
Papaic digest of soybean ..... 5.0 gm.  
Sodium chloride ..... 5.0 gm.  
Agar ..... 15.0 gm.  
Distilled water, q.s ..... 1,000.0 ml.  
pH 7.3 after sterilization .....

(37) *Medium 37.*

Pancreatic digest of casein: 17.0 gm.  
Soybean peptone: 3.0 gm.  
Dextrose: 2.5 gm.  
Sodium chloride: 5.0 gm.  
Dipotassium phosphate: 2.5 gm.  
Distilled water, q.s.: 1,000.0 ml.  
pH 7.3 after sterilization.

(38) *Medium 38.*

Peptone: 15.0 gm.  
Papaic digest of soybean meal: 5.0 gm.  
Sodium chloride: 4.0 gm.  
Sodium sulfite: 0.2 gm.  
L-cystine: 0.7 gm.  
Dextrose: 5.5 gm.  
Agar: 15.0 gm.  
Distilled water, q.s.: 1,000.0 ml.  
pH 7.0 after sterilization.

[39 FR 18944, May 30, 1974, as amended at 40 FR 52004, Nov. 7, 1975; 42 FR 14092, Mar. 15, 1977; 47 FR 9396, Mar. 5, 1982; 47 FR 22514, May 25, 1982]

**§ 436.103 Test organisms.**

(a) *Preparation of test organism suspensions.* For each test organism listed in the following table, select the media (as listed by medium number in § 436.102(b)), incubation period of the Roux bottle, suggested dilution factor, and suggested storage period for the particular test organism and proceed by the appropriate method described in paragraph (b) of this section. Test organism letters A through K, M, and N correspond to those used in "Outline of Details for Official Microbiological Assays of Antibiotics," A. Kirshbaum and B. Arret, "Journal of Pharmaceutical Sciences," Vol. 56, No. 4, p. 512 (April 1967), which is incorporated by reference. Copies are available from the American Pharmaceutical Association, 2215 Constitution Ave. NW., Washington, DC 20037, or available for inspection at the Office of the Federal Register, 800 North Capitol Street, NW., suite 700, Washington, DC.

Test organisms	Method used	Medium used for the—		Incubation period of Roux bottle	Suggested dilution factor	Suggested storage period of suspensions under refrigeration
		Slants	Roux bottles			
Test organism A— <i>Staphylococcus aureus</i> (ATCC 6538P) <sup>2</sup> .	1	1	1	24 hours	1:20	1 week.
Test organism B— <i>Micrococcus luteus</i> (ATCC 7468) <sup>2</sup> .	1	1	1	24 hours	1:30	2 weeks.
Test organism C— <i>Micrococcus luteus</i> (ATCC 9341) <sup>2</sup> .	1	1	1	24 hours	1:40	2 weeks.
Test organism D— <i>Staphylococcus epidermidis</i> (ATCC 12228) <sup>2</sup> .	1	1	1	24 hours	1:14	1 week.