

uninoculated broth prepared as described in the applicable method for the antibiotic being assayed.

[39 FR 18944, May 30, 1974, as amended at 41 FR 34743, Aug. 17, 1976]

#### § 436.101 Solutions.

(a) Antibiotic assay solutions are prepared as follows (solution numbers 1, 2, 3, 4, and 6 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. 222), which is incorporated by reference. Copies are available from the Medical Encyclopedia Inc., 30 East 60th St., New York, NY 11220, or available for inspection at the Office of the Federal Register, 800 North Capitol Street, NW., suite 700, Washington, DC.

(1) *Solution 1 (1 percent potassium phosphate buffer, pH 6.0).*

Dibasic potassium phosphate: 2.0 gm.  
Monobasic potassium phosphate: 8.0 gm.  
Distilled water, q.s: 1,000.0 ml.

Adjust with 18N phosphoric acid or 10N potassium hydroxide to yield a pH 5.95 to 6.05 after sterilization.

(2) *Solution 2 (citrate buffer solution pH 6.3).*

Citric acid: 13.2 gm.  
Sodium hydroxide: 7.06 gm.  
Sodium citrate: 97.0 gm.  
Distilled water, q.s: 1,000.0 ml.

Adjust with 10 percent citric acid solution or 10N sodium hydroxide to yield pH 6.2 to 6.4 after sterilization.

(3) *Solution 3 (0.1M potassium phosphate buffer, pH 8.0).*

Dibasic potassium phosphate: 16.73 gm.  
Monobasic potassium phosphate: 0.523 gm.  
Distilled water, q.s: 1,000.0 ml.

Adjust with 18N phosphoric acid or 10N potassium hydroxide to yield a pH 7.9 to 8.1 after sterilization.

(4) *Solution 4(0.1M potassium phosphate buffer, pH 4.5).*

Monobasic potassium phosphate: 13.6 gm.  
Distilled water, q.s: 1,000.0 ml.

Adjust with 18N phosphoric acid or 10N potassium hydroxide to yield a pH 4.45 to 4.55 after sterilization.

(5) [Reserved]

(6) *Solution 6 (10 percent potassium phosphate buffer, pH 6.0).*

Dibasic potassium phosphate: 20.0 gm.

Monobasic potassium phosphate: 80.0 gm.  
Distilled water, q.s: 1,000.0 ml.

Adjust with 18N phosphoric acid or 10N potassium hydroxide to yield a pH 5.95 to 6.05 after sterilization.

(7)-(9) [Reserved]

(10) *Solution 10 (0.2M potassium phosphate buffer, pH 10.5).*

Dibasic potassium phosphate: 35.0 gm.  
10 N potassium hydroxide: 2.0 ml.  
Distilled water, q.s: 1,000.0 ml.

Adjust with 18N phosphoric acid or 10N potassium hydroxide to yield a pH 10.4 to 10.6 after sterilization.

(11) *Solution 11 (10 percent potassium phosphate buffer, pH 2.5).*

Monobasic potassium phosphate: 100.0 gm.  
Concentrated hydrochloric acid: 0.2 ml. (approximately).  
Distilled water, q.s: 1,000.0 ml.

Adjust with 18N phosphoric acid or 10N potassium hydroxide to yield a pH 2.0 to 2.8 after sterilization.

(12) *Solution 12 (10 percent potassium phosphate buffer, pH 7.0).*

Monobasic potassium phosphate: 100.0 gm.  
Distilled water, q.s: 1,000.0 ml.

Adjust with 18N phosphoric acid or 10N potassium hydroxide to yield a pH 6.95 to 7.05 after sterilization.

(13) *Solution 13 (0.01N methanolic hydrochloric acid).*

1.0N hydrochloric acid: 10.0 ml.  
Methyl alcohol, q.s: 1,000.0 ml.

(14) *Solution 14 (2 percent sodium bicarbonate solution).*

Sodium bicarbonate: 20.0 gm.  
Distilled water, q.s: 1,000.0 ml.

Prepare daily.

(15) *Solution 15 (80 percent isopropyl alcohol solution).*

Isopropyl alcohol: 800.0 ml.  
Distilled water, q.s: 1,000.0 ml.

(16) *Solution 16 (0.1 M potassium phosphate buffer, pH 7.0).*

Dibasic potassium phosphate: 13.6 gm.  
Monobasic potassium phosphate: 4.0 gm.  
Distilled water, q.s.: 1,000.0 ml.

Adjust with 18 N phosphoric acid or 10 N potassium hydroxide to yield a pH 6.8 to 7.2 after sterilization.

(17) *Solution 17 (5 percent methyl alcohol in 1 percent potassium phosphate buffer, pH 6.0).*

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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.