

§ 436.35

21 CFR Ch. I (4–1–98 Edition)

(f) *Method 6.* Proceed as directed in paragraph (a) of this section, except dilute sample with 0.05*N* sodium hydroxide (diluent 9).

(g) *Method 7.* Proceed as directed in paragraph (a) of this section, except dilute sample with sodium carbonate solution (diluent 13).

(h) *Method 8.* Proceed as directed in paragraph (a) of this section, except inject a test dose of 2.0 milliliters of the diluted sample per kilogram of rabbit weight.

(i) *Method 9.* Proceed as directed in paragraph (a) of this section, except dilute sample with pyrogen-free sodium carbonate solution (diluent 15).

(j) *Method 10.* Proceed as directed in paragraph (a) of this section, except dilute the sample with sodium carbonate solution (diluent 16).

[39 FR 18944, May 30, 1974, as amended at 40 FR 51625, Nov. 6, 1975; 45 FR 22921, Apr. 4, 1980; 50 FR 48397, Nov. 25, 1985; 53 FR 13401, Apr. 25, 1988]

§ 436.35 Depressor substances test.

Proceed as directed in the USP XX depressor substances test. Prepare the sample test solution as follows: For each antibiotic listed in the table below, select the appropriate diluent and test dose (concentration and volume). If the product is packaged for dispensing and is in a combination package with a container of diluent, dilute the product as directed in the labeling.

Antibiotic	Diluent ¹	Concentration of test solution ²	Volume of test solution to be injected ³
Bleomycin sulfate	4	40.5	1.0
Capreomycin sulfate	4	3.0	1.0
Chlortetracycline hydrochloride	3	5.0	.6
Clindamycin phosphate	4	5.0	1.0
Daunorubicin hydrochloride ...	4	1.5	1.0
Dihydrostreptomycin sulfate ...	4	3.0	1.0
Doxorubicin hydrochloride	4	1.5	1.0
Doxycycline hyclate	4	5.0	1.0
Lincomycin hydrochloride monohydrate	4	3.0	1.0
Minocycline hydrochloride	4	5.0	.6
Plicamycin	3	0.050	1.0
Mitomycin	4	0.050	1.0
Oxytetracycline ⁵	3	5.0	.6
Oxytetracycline hydrochloride	4	5.0	.6
Rolitetraacycline	4	5.0	.6
Rolitetraacycline nitrate	4	5.0	.6
Sodium colistimethate	4	3.0	1.6
Spectinomycin hydrochloride	4	15.0	1.0
Streptomycin sulfate	4	3.0	1.0

Antibiotic	Diluent ¹	Concentration of test solution ²	Volume of test solution to be injected ³
Tetracycline hydrochloride	4	5.0	.6
Tetracycline phosphate ⁵	3	5.0	.6
Vidarabine monohydrate ⁵	4	1.0	1.0

¹ Diluent number as listed in sec. 436.31(b).

² Milligrams of activity per milliliter.

³ Milliliters per kilogram of body weight.

⁴ The concentration of the test solution is expressed in units per milliliter in lieu of milligrams of activity per milliliter.

⁵ To prepare the test solution, proceed as directed in the individual section of the antibiotic drug regulation in this chapter for the antibiotic to be tested.

[46 FR 60568, Dec. 11, 1981, as amended at 46 FR 61071, Dec. 15, 1981; 49 FR 5096, Feb. 10, 1984]

Subpart D—Microbiological Assay Methods

§ 436.100 Laboratory equipment.

Equipment should be selected which is adequate for its intended use and should be thoroughly cleansed after each use to remove any antibiotic residues. The equipment should be kept covered when not in use. Clean glassware intended for holding and transferring the test organisms should be sterilized in a hot air oven at 200–220° C. for 2 hours. Volumetric flasks, pipettes, or accurately calibrated diluting devices should be used when diluting standard and sample solutions.

(a) *Microbiological agar diffusion assay*—(1) *Cylinders.* Use stainless steel cylinders with an outside diameter of 8 millimeters (±0.1 millimeter), an inside diameter of 6 millimeters (±0.1 millimeter), and a length of 10 millimeters (±0.1 millimeter).

(2) *Plates.* Plastic or glass Petri dishes may be used, having dimensions of 20 by 100 millimeters. Covers should be of suitable material.

(b) *Microbiological turbidimetric assay*—(1) *Tubes.* Tubes which give satisfactory results and have uniform length and diameter should be used. If reusable tubes are employed, care must be taken to remove not only all antibiotic residues from the previous test but also all traces of cleaning solution.

(2) *Colorimeter.* Use a suitable photoelectric colorimeter at a wavelength of 530 millimicrons. Set the instrument at zero absorbance with clear,

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).*