**Reagents, Test Solutions, Etc.**

Reagents, test solutions, etc. are used for the tests in this standard. In addition to those specified herein, reagents and test solutions shall proceed as directed in Reagents, Test Solutions, etc. specified in the General Tests, Processes and Apparatus in the *Japanese Pharmacopoeia*. In this standard, the reagents noted with [JP], [JP monograph], [Special grade], or [1st grade] represent that they conform to the Reagents, Test solutions specified in the General Tests, Processes and Apparatus in the *Japanese Pharmacopoeia*, to the pharmaceuticals specified in the Official Monographs in the same, and to the reagents noted with the Special grade or 1st grade in the Japanese Industrial Standards. The reagent is marked with an asterisk (\*) in the name of the reagent shall meet the suitable quality for their intended uses.

A

Acetic acid (100) [Glacial acetic acid, Special grade]

Acetone [Special grade]

Acetylacetone [Special grade]

Acriflavine\*

0.2 w/v% acriflavine

Dissolve 2.0 g of acriflavine in water to make 1,000 mL and sterilize at 121 ℃ for 15 minutes.

Agar [Agar, JP monograph]

Agar (for sterility test)

An agar that conforms to the following specifications shall be used:

(1) Nitrogen content: not more than 0.5%.

(2) When conducting a test by the method specified in the Japanese Industrial Standards, the jelly strength at a concentration of 1.5% is 300 to 500 g/cm2.

Agar medium containing 0.02 w/v% acriflavine

Add acriflavine to nutrient agar medium so that it is contained at a rate of 0.02 w/v%.

Agar medium containing 10 vol% horse serum

Add horse serum in nutrient agar medium so that it is contained at a concentration of 10 vol%.

Aluminum (III) chloride hexahydrate [Aluminum chloride, Special grade]

4-Aminobenzoic acid [p-Aminobenzoic acid, Special grade]

2-Amino-2-hydroxymethyl-1,3-propanediol [tris (hydroxymethyl) aminomethane, Special grade]

Ammonia water (28) [Ammonia water, Special grade]

Ammonium acetate [Special grade]

Ammonium thiocyanate [Special grade]

Anhydrous calcium chloride See calcium chloride, anhydrous.

Anthrone [Special grade]

L-arginine monohydrochloride [Special grade]

B

Bacto peptone\*

Barbital [JP monograph]

Barbital sodium salt [JP]

Barium chloride [Special grade]

Benzylpenicillin potassium [JP monograph]

N,N-Bis(2-hydroxyethyl)-2-aminoethanesulfonic acid\*

Boric acid [JP monograph]

Bovine serum albumin

Bovine serum albumin is a pale-yellow or pale-brown powder purified from bovine serum by a method that does not affect albumin and other plasma proteins, and it conforms to the following specifications:

(1) The 10 w/v% solution is clear, and its pH ranges from 5.0 to 5.5.

(2) When conducting a test using electrophoresis, albumin must be not less than 97% of total protein.

5.5 w/v% bovine serum albumin solution

Dissolve 5.5 g of bovine serum albumin in water to make 100 mL.

Bromocresol green [Special grade]

C

Calcium chloride, anhydrous [Special grade]

Calcium chloride dihydrate [Calcium chloride, Special grade]

3 w/v% calcium chloride solution

Dissolve 3.0 g of calcium chloride dihydrate in water to make 100 mL and sterilize at 121℃ for 15 minutes.

Carbon tetrachloride [Special grade]

Casein peptone

Description: Casein peptone is a grayish yellow powder. It has a characteristic odor but has no putrefactive odor. It is soluble in water but insoluble in ethanol (95) or diethyl ether.

Digestibility: Dissolve 1 g of casein peptone in 10 mL of water and use as the sample solution for conducting the following tests:

(1) On 1 mL of sample solution, superimpose 0.5 mL of solution prepared by adding 1 mL of acetic acid (100) to 10 mL of dilute ethanol: no ring or precipitate is produced at the zone of contact. When mixing this solution, no turbidity is produced.

(2) To 1 mL of sample solution add 4 mL of saturated zinc sulfate solution: a small amount of precipitate is produced.

(3) Filter the mixture prepared in (2). To 1 mL of the filtrate add 3 mL of water and 0.2 mL of bromine test solution: a red-violet color develops.

Casein peptone (for sterility test)

In addition to its specifications, casein peptone used conform to the following specifications:

(1) The 2 w/v% solution is pale yellow clear, and its pH ranges from 6.5 to 7.0.

(2) When measured by the Van Slyke method and Kjeldahl method, amino nitrogen is 25% to 50% of total nitrogen.

(3) When measured by the microbioassay or other assays, the content of tryptophan is not less than 1.5%.

(4) Prepare the following five types of media A–E, adjust the pH to 7.2 to 7.4, and conduct the following tests a–e: the media conform to the specifications:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Medium | Sample | Sodium chloride | Others | Water |
| A | 2.0 g | 0.5 g | – | 100 mL |
| B | 1.0 g | 0.5 g | – | 100 mL |
| C | 0.1 g | 0.5 g | – | 100 mL |
| D | 1.0 g | 0.5 g | Glucose 0.5 g | 100 mL |
| E | 2.0 g | 0.5 g | Agar 1.5 g | 100 mL |

a Fermentable carbohydrates test

To 5 mL of Medium A, add an appropriate amount of phenol red test solution, put a Durham tube, inoculate a loopful of *Escherichia coli* incubated at approximately 36 ºC for 24 hours, and incubate it at 35ºC to 37°C for 48 hours: the bacteria grow well, and no acid or gas is produced.

b Hydrogen sulfide production test

To 5 mL of Medium B, inoculate *Salmonella typhi*, place a piece of lead acetate paper between the cotton stopper and the mouth of the test tube at a height of about 5 cm above the medium surface, and incubate at 35ºC to 37°C for 24 hours: the bacteria grow well, and when incubated for 48 hours, the piece of paper shows a marked dark brown color.

c Indole production test

Inoculate *Escherichia coli* in 5 mL of Medium C at 35ºC to 37°C for 24 hours: *Escherichia coli* grows well; layer 0.5 mL of Indole test solution 0.5 mL: a red-violet color develops clearly at the zone of contact.

d Acetyl methyl carbinol production test

Inoculate *Klebsiella pneumoniae* in 5 mL of Medium D at 35ºC to 37 ºC for 24 hours: *Klebsiella pneumoniae* grows well; add 5 mL of 10 w/v% sodium hydroxide solution, shake, and allow to stand at approximately 25 ºC for 5 hours: a pale-red color develops.

e Bacterial growth-promoting ability test

(a) Puncture *Brucella melitensis* in a stab medium of Medium E at 35ºC to 37 ºC for 48 hours: bacteria grow along the puncture line.

(b) Inoculate *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella Typhi,* *Pseudomonas aeruginosa*, *Staphylococcus aureus,* and *Staphylococcus epidermidis* in a slant medium of Medium E at 35ºC to 37 ºC for 24 hours: all bacteria grow well.

(c) To Medium E, add defibrinated blood from a rabbit so that it is contained at a concentration of approximately 5 vol%, inoculate *Streptococcus pneumoniae* or β-hemolytic *Streptococcus pneumoniae*, mix well, fix on a plate, and incubate at 35ºC to 37 ºC for 48 hours: both bacteria grow well, showing a characteristic α or β- hemolytic zone.

(d) To Medium E, add defibrinated bovine blood so that it contained at a concentration of approximately 10 vol%, fix on a plate, heat at 80ºC to 90 ºC until the medium turns to chocolate brown in color, grow *Neisseria gonorrhoeae*, and incubate gas at 35ºC to 37 ºC for 48 hours: the bacteria grow well when incubated in an incubator maintaining 10% carbon dioxide.

Chicken water\*

Chloroform [Special grade]

Copper (II) sulfate pentahydrate [Copper sulfate, Special grade]

L-cystine [Special grade]

L-cysteine hydrochloride monohydrate [Special grade]

1 w/v% L-cysteine monohydrochloride test solution

Dissolve 1.0 g of L-cysteine hydrochloride monohydrate in water to make 100 mL.

D

Dibasic sodium phosphate anhydrous See sodium dihydrogen phosphate, anhydrous.

Dipotassium hydrogen phosphate [Dipotassium hydrogen phosphate, Special grade]

Disodium hydrogen phosphate dodecahydrate [Disodium hydrogen phosphate 12-water, Special grade]

Dithizone [Special grade]

E

Eagle’s MEM

Dissolve a dried product of suitable quality according to the direction and sterilize.

Earle's solution

Calcium chloride dihydrate 0.2 g

Potassium chloride 0.4 g

Magnesium sulfate heptahydrate 0.2 g

Sodium chloride 6.8 g

Sodium hydrogen carbonate 2.2 g

Sodium dihydrogen phosphate dihydrate 0.163 g

Glucose 1.0 g

Phenol red 0.05 g

Dissolve all the above-listed ingredients in water to make 1,000 mL, and filter sterilize.

A dried product of suitable quality may be used if it is prepared by dissolving according to the direction and sterilized.

Ethanol (95) [ethyl alcohol, Special grade]

Ethanol (99.5) [Anhydrous ethanol, Special grade]

90 vol% ethanol

Add water to 180 mL of ethanol (99.5) to make 200 mL.

F

Formalin [JP monograph]

F10 medium

Dissolve the dried F10 medium of suitable quality according to the direction and sterilize.

G

Gelatin [JP monograph]

Glacial acetic acid See acetic acid (100).

Glucosamine\*

Glucose [JP monograph]

Glycerin [Special grade]

H

Hank’s solution

Sodium chloride 8.0 g

Potassium chloride 0.4 g

Disodium hydrogen phosphate dodecahydrate 0.06 g

Glucose 1.0 g

Magnesium chloride hexahydrate 0.2 g

Calcium chloride dihydrate 0.14 g

Sodium hydrogen carbonate 0.35 g

Phenol red 0.02 g

Dissolve all the above-listed ingredients in water to make 1,000 mL, filter sterilize.

A dried product of suitable quality may be used if it is prepared by dissolving according to the direction and sterilized.

Heart infusion broth

Hydrochloric acid [Special grade]

Hydrogen peroxide (30) [Hydrogen peroxide solution, Special grade]

I

Indole test solution\*

K

Kaolin [JP monograph]

25 w/v% kaolin solution

Suspend 25 g of kaolin in water to make 100 mL.

L

Lactalbumin hydrolysate\*

Lactose\*

Lactose agar\*

Liquid paraffin [Light liquid paraffin, JP monograph]

M

Macrogol 4,000 [JP monograph]

Macrogol 6,000 [JP monograph]

Magnesium chloride hexahydrate [Magnesium chloride, Special grade]

Magnesium sulfate heptahydrate [Magnesium sulfate, Special grade]

Malachite green oxalate [Malachite green (oxalate), Special grade]

2 w/v% malachite green solution

Dissolve 2.0 g of malachite green oxalate in water to make 100 mL.

Meat extract broth\*

Meat peptone\*

Meat water\*

Methyl red [Special grade]

Methyl red test solution

Dissolve 0.1 g of methyl red in ethanol (95), and filter if necessary.

Monosodium glutamate\*

Mucin\*

N

Neutral red [Special grade]

1 w/v% β-Nicotinamide-adenine dinucleotide [oxidized form] solution\*

Nitric acid [Special grade]

4-Nitroaniline [p-nitroaniline, 1st grade]

Nutrient agar medium

Dissolve 15 g of agar in 1,000 mL of nutrient broth with the aid of heat, add water to make up for the loss, adjust the pH to between 6.4 and 7.0, and filter. Dispense the filtrate and sterilize by autoclaving at 121℃ for 15 minutes.

Nutrient broth

Dissolve 5 g of meat extract and 10 g of peptone in 1,000 mL of water by gentle heating. Adjust the pH of the mixture between 6.4 and 7.0 after sterilization, cool, add water to make up for the loss, and filter. Sterilize the filtrate by autoclaving for 30 minutes at 121℃.

Nutrient broth containing 10 vol% horse serum

Add horse serum in nutrient broth so that it is contained at a concentration of 10 vol%.

P

Peptone\*

Peptone for toxins\*

Peroxidase-conjugated anti-mouse immunoglobulin\*

Phenol [Special grade]

Phenol red [Special grade]

0.2 w/v% phenol red solution

Dissolve 2.0 g of phenol red in water to make 1,000 mL.

Phosphate-buffered saline (pH 7.4–7.45)

Dissolve 8.0 g of sodium chloride, 0.2 g of potassium chloride, 1.15 g of dibasic sodium phosphate anhydrous, and 0.2 g of potassium dihydrogen phosphate in water to make 1,000 mL and sterilize.

1/60 mol/L phosphate-buffered saline (pH 7.0)

Dissolve 14.45 g of dibasic sodium phosphate anhydrous, 8.83 g of potassium dihydrogen phosphate, and 85.0 g of sodium chloride in water to make 10,000 mL and sterilize.

Phosphate-buffered saline containing 2 vol% horse serum

Add horse serum in phosphate-buffered saline so that it is contained at a concentration of 2 vol%.

1/60 mol/L phosphate-buffered saline containing 0.2 w/v% gelatin (pH 7.0)

Add gelatin in 1/60 mol/L phosphate-buffered saline so that it is contained at a concentration of 0.2 w/v%, heat until dissolved and sterilize.

Phosphate-buffered saline containing 0.5 w/v% phenol (pH 7.0)

1.45 g of potassium dihydrogen phosphate, 15.28 g of disodium hydrogen phosphate dodecahydrate, 4.8 g of sodium chloride, and 5.0 g of phenol in water to make 1,000 mL, and filter sterilize.

Phosphomolybdic acid n-hydrate [Phosphomolybdic acid, Special grade]

Phosphorus (V) oxide [Phosphorus (V) oxide, Special grade]

Physiological saline [JP monograph]

Physiological saline containing 0.3 vol% formalin

Add 3.0 mL of formalin to physiological saline to make 1,000 mL.

Physiological saline containing 50 vol% glycerin

Add 50 mL of physiological saline in 50 mL of glycerin.

Physiological saline containing 2 vol% horse serum

Add horse serum in physiological saline so that it is contained at a concentration of 2 vol%.

Physiological saline containing 0.1 w/v% magnesium sulfate

Dissolve 1.0 g of magnesium sulfate heptahydrate in physiological saline to make 1,000 mL and sterilize.

Physiological saline containing 0.5 w/v% phenol

Dissolve 5.0 g of phenol in physiological saline to make 1,000 mL.

Polysorbate 80 [JP monograph]

Potassium chloride [Special grade]

Potassium dihydrogen phosphate [Potassium dihydrogen phosphate, Special grade]

Potassium periodate [Potassium periodate, Special grade]

Potassium sulfate [JP monograph]

Potato extract\*

R

RDE\*

Resazurin [JP]

0.1 w/v% resazurin solution

Dissolve 1.0 g of resazurin in water to make 1,000 mL.

S

Silica gel [JP]

Skimmed milk powder\*

Sodium acetate trihydrate [Sodium acetate (trihydrate), Special grade]

Sodium azide

Description: Sodium azide is white or practically white crystals. It is easily soluble in water, and practically insoluble in diethyl ether. The solution in water is alkaline.

Qualitative test: When treated with iron (III) nitrate test solution, sodium azide solution turns to reddish brown in color. When sodium azide is heated in a colorless flame, the flame turns yellow in color.

Content: Not less than 97.0%

10 w/v% sodium azide solution

Dissolve 10 g of sodium azide in water to make 100 mL.

Sodium carbonate, anhydrous [Anhydrous sodium carbonate, Special grade]

Sodium carbonate decahydrate [Sodium carbonate, Special grade]

Sodium carboxymethyl cellulose [JP monograph]

Sodium chloride [Special grade]

Sodium dihydrogen phosphate, anhydrous [Disodium hydrogen phosphate anhydrous, Special grade]

Sodium dihydrogen phosphate dihydrate [Sodium dihydrogen phosphate, Special grade]

Sodium hydrogen carbonate [Special grade]

Sodium hydroxide [Special grade]

Sodium nitrite [Special grade]

Sodium thioglycolate [Special grade]

Soybean-casein digest agar

Dissolve a dried soybean-casein digest agar of suitable quality according to the direction and sterilize.

Soy peptone\*

Stilbazo

(1) A black-brown powder.

(2) Shows yellow at pH 3–7, orange at pH 9 and red at pH 11 when dissolved in water. No insoluble matter is produced when 50 mg of stilbazo is dissolved in 100 mL of water.

(3) When igniting 0.5 g of sodium hydroxide with 1 mL of stilbazo and weighing, the weight of the residue shall not exceed 10 mg.

(4) The absorbance of 0.002 w/v% solution of stilbazo at 410 nm must be not less than 0.7.

(5) Add water to 5 mL of 0.05 w/v% solution of stilbazo, 10 mL of 0.0001 mol/L aluminum chloride and 10 mL of 1 mol/L of acetate buffer solution to make 100 mL. The absorbance of the diluted solution at wavelength 505 nm and optical path length 10 mm is higher by 0.42 or more than that of stilbazo alone.

Sulfuric acid [Special grade]

T

Thallium acetate\*

Thiamine chloride [JP monograph]

Thimerosal C9H9HgNaO2S

Description: Thimerosal is a white or pale-yellow crystalline powder, having a slight characteristic odor. The pH of the solution at a concentration of 1.0 g/100 mL ranges from 6.0 to 7.0.

Purity

(1) Clarity and color of solution: Colorless and clear (solution at a concentration of 1.0 g/10 mL)

(2) Diethyl ether-soluble substances: Weigh precisely about 0.5 g of powdered thimerosal, put in a 50-mL glass-stoppered conical flask, add 20 mL of anhydrous diethyl ether, stopper, shake for 10 minutes, filter in a weight-known beaker through filter paper previously washed with diethyl ether, wash the residues with 5 mL of anhydrous diethyl ether, combine the filtrate and washings, evaporate the combined solution on a water bath, and dry in a desiccator (in vacuum, silica gel) for 24 hours: the mass of the residue is not more than 0.60%.

(3) Other soluble mercury salt: Dissolve 0.10 g of thimerosal in 10 mL of water, add 3 drops of acetic acid (31) in 5 mL of this solution: a white precipitate is produced. To this solution, add 1 drop of sodium sulfide, and allow to stand for 10 minutes: no dark color develops in the solution.

(4) Readily carbonizable substances: Take 0.20 g of thiomersal and conduct the test as specified the General Tests, Processes and Apparatus in the *Japanese Pharmacopoeia*. The solution has no more color than that of J. the matching fluid for color. The test must be conducted at a standard temperature.

Loss on drying: Not more than 0.5% (1 g, in vacuum, silica gel, 5 hours)

Content: Not less than 98.0%

Assay: Weigh precisely about 0.3 g of previously dried thimerosal for the assay, put in a 300 mL Kjeldahl flask, add 10 mL of sulfuric acid and 4 mL of fuming nitric acid, heat gently on a sand bath, and gradually increase the heat until the contents in the flask become almost colorless and white smoke is produced. After cooling, transfer the contents with 100 mL of water to a beaker and heat on a water bath for 15 minutes while occasionally shaking. Then add 0.5 g of urea, shake, and add potassium permanganate test solution dropwise until a slight pale red color develops. After cooling, add hydrogen peroxide test solution dropwise until the red color of the solution disappears, and titrate with 0.1 mol/L ammonium thiocyanate solution (indicator: 2 mL of ammonium iron (III) sulfate test solution).

1 mL of 0.1 mol/L ammonium thiocyanate solution = 20.240 mg C9H9HgNaO2S

Storage: Preserve in a light-resistant tight container.

Thiourea [Special grade]

Tin (II) chloride dihydrate [Stannous chloride, Special grade]

Trichloroacetic acid [trichloroacetic acid, Special grade]

Tris-HCl buffer solution

Dissolve 20.5 g of 2-Amino-2-hydroxymethyl-1,3-propanediol, 13.6 g of sodium acetate trihydrate, 2.2 g of calcium chloride dihydrate and 0.68 g of zinc chloride in 700 mL of water, add hydrochloric acid, and adjust the pH to 7.4 to make 1,000 mL in total.

Tris(hydroxymethyl)aminomethane See 2-Amino-2-hydroxymethyl-1, 3-propanediol.

Trypsin\*

Tryptose phosphate broth\*

V

Veronal-buffered saline (pH 7.2)

Add 85.0 g of sodium chloride, 5.75 g of barbital and 3.75 g of barbital sodium salt to water to make 1,000 mL, and filter sterilize.

Veronal-buffered saline containing 1 w/v% gelatin (pH 7.5)

Add gelatin in veronal-buffered saline so that it is contained at a concentration of 1 w/v%, heat until dissolved, and sterilize.

Y

Yeast extract

A peptone-like substance which represents all the soluble product of yeast cells (*Saccharomyces*) prepared under optimum conditions, clarified, and dried by evaporating to a powder. Yeast extract (1 g) represents not less than 7.5 g of yeast. A reddish yellow to brown powder with a characteristic odor. Soluble in water and forms a yellow to brown solution.

(1) The nitrogen content of yeast extract is 7.2% to 9.0%.

(2) The loss on drying at 105℃ at constant mass of yeast extract is not more than 5%.

(3) The residue of yeast extract when ignited is not more than 15%.

(4) Heat 5 w/v% yeast extract solution until boiling: no precipitate is produced.

(5) Chloride (as sodium chloride): not more than 5%.

Z

Zinc chloride [Special grade]