

JIS

JAPANESE INDUSTRIAL STANDARD

General rules for biochemical reagents

JIS K 8008-1992

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Translated
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JAPANESE INDUSTRIAL STANDARD

J I S

General rules for biochemical reagents

K 8008-1992

1. Scope This Japanese Industrial Standard specifies the matters commonly related to the reagents⁽¹⁾ for biochemistry.

Note ⁽¹⁾ The biochemical reagents to which this Standard applies are amino acids, coenzymes, enzymes, nucleic acid, enzyme substrates, biochemical buffers, carbohydrates, and proteins.

Remarks: The standards cited in this Standard are shown in Attached Table 1.

2. General matters

- 2.1 Definition of terms The definition of main terms used in this Standard shall be as follows, besides those defined in JIS K 3600.

- (1) biochemical reagents Biochemical reagents generally mean chemical materials which are used for detection or determination of materials carried out biochemically or chemically, or for the synthetic experiments of material or the measurements of physical characteristics of materials, in the field of biotechnology and its application, that is, gene manipulation, cell fusion, tissue · cell culture, and so on.

- (2) endotoxin This is a component of cell membrane of gram-negative bacillus, which has activities such as exothermic activity and coagulation on hemacyte component of limuli.

- (3) sterilization To destroy or eliminate all microorganisms existing in objects.

Remarks: The sterilization methods are classified into heating method, filtration method, radiation method, gas method, and medical-liquid method.

- (4) pasteurization To destroy the microorganisms existing in objects.

Remarks: The pasteurization methods are classified into heating method, radiation method, gas method, and medical-liquid method.

- (5) removal of bacteria To remove microorganisms from objects by filtration or washing.

- (6) enzyme unit The enzyme unit means the amount of enzyme by which unit quantity of substrate is transformed for specified time under the specified condition.

- (7) enzyme activity Degree of catalytic activity of enzyme, that is, the reaction rate of enzyme.

Remarks: The degree of enzyme activity is expressed with enzyme unit.

- (8) specific activity Enzyme activity performed by unit quantity of enzyme.

- (9) deactivation of enzyme This means to make the enzyme activity decrease or vanish, or means decrease or vanishing of enzyme activity by itself.

Remarks: Enzyme is deactivated by not only thermal deactivation, acid deactivation, but also by adding · mixing of enzyme inhibitor, removal · vanishing of auxiliary factor, and so on.

- (10) aseptic condition The condition of an object where there is no microorganisms capable of growing.

Remarks: Aseptic condition is created by sterilization operation, and shall be tested by asepsis method and the like.

- (11) index bacteria Bacteria used as an index for the selection of sterilizing condition or confirmation of sterilizing effect.

- (12) LAL LAL is the freeze-dried product prepared from the extracted hemacyte of limuli which can be activated and gelled by a very small amount of endotoxin or can hydrolyze coloring synthetic substrate, and therefore is used for detection or determination of endotoxin existing in sample in case of an endotoxin test.

- (13) coloring synthetic substrate (for endotoxin test) This is the freeze-dried synthetic substrate containing chromophore, which can be hydrolyzed by coagulating enzyme contained in activated LAL and then can isolate the chromophore, when endotoxin is determined by colorimetry.

- (14) pyrogen test This is the test to confirm the existence of pyrogenic substance, using a rabbit, which the bodily temperature of homiothermic animals is elevated abnormally by its dosing in the sample.

- (15) endotoxin test This reaction is for the detection or determination of endotoxin based on either the reaction causing the gelling after LAL has been activated by endotoxin which derived from gram-negative bacillus, or the hydrolysis of peptide by means of coagulating enzyme contained in the gelling reaction cascade.

- (16) superfiltration The filtering process using a membrane filtering device such as a reverse osmosis membrane, ultrafiltration membrane, or their combination which has an ability to remove such microorganisms as virus, mycoplasma, bacteria, fungus, and endotoxin with the method of crossflow filtration or the like.

- (17) deactivation of endotoxin This means eliminating the activity of existing endotoxin.

Remarks: Deactivation is done by heating method, ultrafiltration method, radiation method, or the like.

- (18) potency of endotoxin This means the activity of endotoxin measured by endotoxin test.

The amount of buffer solution or endotoxin test water to be added shall be decided at every container (one vial) for LAL of each lot, and the potency after adding shall be marked on every container (one vial). Hereafter this value shall be designated as LAL marked potency.

2.2 Designation Designation shall be as follows besides those specified in JIS K 8001.

- (1) In case of enzyme, use the name recommended by International Union of Biochemistry (IUB). When there is no recommended name, use the customary name prevailed most popularly. If necessary, attach systematic name and enzyme number.
- (2) In case of proteins or the others, principally follow the prescription of International Union of Pure and Applied Chemistry (IUPAC) or IUB, but when these cannot be applied, use the customary name which has been recognized by related academic societies and prevailed most popularly.
- (3) In case of enzymes and proteins, specify its origin.

2.3 Units and symbols Units and symbols shall be as follows besides using SI.

If necessary, they shall be specified in a respective standard.

- (1) **Katal (kat)** One katal (kat) is the amount of enzyme which transforms 1 mol of substrate per 1 s at the optimal condition.

Remarks: The unit of μkat , nkat , or pkat can also be used. Specific activity is expressed by kat/kg protein, and the relation between conventional enzyme unit (U) and kat is $1 \text{ kat} = 6 \times 10^7 \text{ U}$.

Informative reference: Katal (kat) is a new enzyme unit defined in the Third (1972) Recommendation of Enzyme Committee in International Union of Biochemistry.

- (2) **Unit (U)** One Unit (U) is the amount of enzyme which transforms 1 μmol of substrate per 1 min at the optimal condition.

Remarks: Specific activity is expressed by U/mg protein, and the relation between newly decided enzyme unit katal (kat) and Unit is $1 \text{ U} = 16.67 \text{ nkat}$.

Informative reference: Unit (U) is conventional enzyme unit defined in the First (1961) Recommendation of Enzyme Committee in International Union of Biochemistry.

- (3) **Endotoxin unit (EU)** Endotoxin unit (EU) equals to the potency of endotoxin contained in 0.2 ng of US Standard Endotoxin (*E. coli* 0113 strain) lot EC-2. $0.2 \text{ ng of endotoxin} = 1 \text{ EU}^{(2)}$.

Note ⁽²⁾ Standard endotoxin in Japan is standardized by lot EC-5, and its endotoxin unit (EU) has been determined according to the endotoxin test specified in the twelfth revised Japanese Pharmacopoeia.

3. Matters in common Matters in common shall be as follows besides those specified in JIS K 0050 and JIS K 8001.

3.1 Test surroundings When there is no specification in respective standard, make the surroundings specified in JIS Z 8703, the standard condition, and besides if necessary, the followings shall be provided for tests.

- (1) Clean room The number of particles, with 0.5 μm or larger size, in the clean room shall be 3500000/ m^3 or less⁽³⁾ when the number of floating particles are measured according to JIS B 9920.

Note ⁽³⁾ This condition corresponds to the cleanliness class 8 specified in JIS B 9920 or higher class.

- (2) Clean bench The clean bench shall be the one specified in JIS B 9922.

Remarks: If necessary, the clean bench equipped with an air curtain shall be used.

- (3) Bioclean room The number of particles, with 0.5 μm or larger size, in the bioclean room shall be 3500/ m^3 or less⁽⁴⁾ when the number of floating particles are measured according to JIS B 9920.

Note ⁽⁴⁾ This condition corresponds to the cleanliness class 5 specified in JIS B 9920 or higher class. The air flow sent into the room shall be positive against ambient atmospheric pressure.

- (4) Safety cabinet Safety cabinet shall never leak out the aerosol generated during testing.

Remarks: The safety cabinet shall be used for the recombinant DNA experiments where physical containment of P2 level⁽⁵⁾ or LS-1 level⁽⁶⁾ or higher level is required, or for the experiments where the pathogen with dangerousness 3a⁽⁷⁾ or higher level is handled. In the case of the experiments likely produce aerosol, the cabinet should be used even when pathogen with dangerousness 2b⁽⁸⁾ is handled.

Notes ⁽⁵⁾ P2 level means the level of physical containment specified in "Guidelines for recombinant DNA experiment" (Decided by Prime Minister on August 27, 1979. Finally revised on September 16, 1987. Hereafter referred to as Guidelines for recombinant DNA experiment) where experimental guideline is shown as follows: when recombinant DNA experiments is carried out by 20 l or smaller size, the safety cabinet shall be established not to leak out aerosol generated in handling recombinant, or the apparatus which has containment capacity corresponding to this cabinet shall be used; the laboratory shall be set inside the building equipped with an autoclave for the disinfection of contaminated and the wastes; besides, the sign showing that P2 level experiment is now carrying out shall be marked at the entrance of the laboratory; and so on.

⁽⁶⁾ LS-1 level means the level of physical containment specified in "Guidelines for recombinant DNA experiment" where experimental guideline is shown as follows: when the large amount culture experiment is carried out by larger size than 20 l, the safety cabinet shall be established, which has been designed to prevent the leakage of recombinant, has been equipped with the apparatus for culture where sterilizing operations can be remotely controlled as it is closed, and can contain the aerosol produced in handling recombinant; alternatively, the apparatus which has containment capacity corresponding to this cabinet shall be provided; the

exhaust gas from the apparatus for culture shall be discharged only through a bacteria-removing filter or the bacteria-removing device corresponding to the filter; besides, the area for the experiment handling a large amount of culture shall be clearly defined; the sign showing that large amount of culture experiment of LS-1 level is now carried out shall be marked at the area of large-amount culture experiment; and so on.

- (7) The pathogen of dangerousness 3a is stipulated in the "Classification criteria of dangerousness of pathogens" in the "Regulation for Safety Control of Pathogens of the National Institute of Health" (Decided by the National Institute of Health on November 5, 1975. Revised on March 24, 1983. Hereafter referred to as "Regulation for safety control of pathogens"), and is specified as either of the followings.
 - (1) The pathogen gives considerable many chances to infect in a laboratory, but even if infected, it will result in slight illness.
 - (2) The pathogen is always found in Japan, but because almost all adults have immunity, there is few chance to be infected in a laboratory. When being infected, however, it may give serious illness.
- (8) The pathogen of dangerousness 2b is stipulated in the "Classification criteria of dangerousness of pathogens" in the "Regulation for safety control of pathogens", and is specified as follows; the infection in a laboratory is nearly prevented as long as ordinary handling procedures of microorganism are carried out. Even if being infected, the illness by the pathogen appears extremely seldom.
- (5) Facilities for radiation The facilities for radiation shall be designed to prevent the outbreak of radiation lesion, when handling radioisotope and the contaminated by radioisotope (hereafter referred to as radioisotope and the like), or the apparatus for radiation emission.

Informative reference: "The law concerning prevention of radiation hazards due to radioisotopes and others" says that the facilities for radiation includes the facility for utilizing, facility for repacking, facility for repacking of waste, facility for storing, facility for set-up of instruments, facility for waste storing, and facility for discarding.

3.2 Water Water shall be specified as follows besides being specified in a respective standard or in 4.3.

3.2.1 Class and quality Water shall be classified into A1 to A4, and their quality shall be specified as shown in Table 1.

Table 1. Quality of water for chemical analysis

Items	Class			
	A1	A2	A3	A4
Conductivity (mS/m) (25°C)	0.5 max.	0.1 ⁽⁹⁾ (¹⁰) max.	0.1 ⁽⁹⁾ max.	0.1 ⁽⁹⁾ max.
Organic carbon (TOC) (mgC/l)	1 max.	0.5 max.	0.2 max.	0.05 max.
Zinc (µgZn/l)	0.5 max.	0.5 max.	0.1 max.	0.1 max.
Silica (µgSiO ₂ /l)		50 max.	5.0 max.	2.5 max.
Chloride ion (µgCl ⁻ /l)	10 max.	2 max.	1 max.	1 max.
Sulfate ion (µgSO ₄ ²⁻ /l)	10 max.	2 max.	1 max.	1 max.
Test by potassium permanganate (ml)(¹¹)		0.5 max.	0.3 max.	0.2 max.

Notes ⁽⁹⁾ This is the value given when the water at outlet of a water purifying apparatus is introduced directly to the detecting element of a conductivity tester and measured.

⁽¹⁰⁾ When such filter as an accurate-class filter is directly connected to the outlet of an ion exchanging device, this value turns into 0.01 mS/m (25°C) or less.

⁽¹¹⁾ This is the volume (ml) of 5 mmol/l potassium permanganate solution consumed when the mixture of 100 ml water, acidified with sulfuric acid, and specified amount of 5 mmol/l potassium permanganate solution is heated for 30 min.

Remarks: The items of each class of water quality mentioned in Table 1 are chosen as typical ones representing the quality of water. Therefore, other items than these can be supplemented or some of these can be eliminated in answer to the kind and object of chemical analysis. The supplementation of the items other than mentioned in Table 1 or the elimination of these shall follow the test method listed on a respective standard.

A1 can be used for washing of implements, etc. A1 is purified through an ion exchange method at final purifying stage, or through the method to be able to get the same quality as this.

A2 can be used for ordinary chemical analysis, etc. A2 is usually purified through an ion exchange method (connected with an accurate-class filter at its outlet part) at its final stage, using A1 water, or through the method to be able to get the same quality as this.

A3 can be used for the preparation of reagent solutions or the chemical analysis of micro-components, etc. A3 is usually purified, using water A1 or water A2, through distilling method at its final purifying stage, or through the method to be able to get the same quality as this.

A4 can be used for the test of organic substance or the chemical analysis of micro-components, etc. A4 is usually purified, using water A1 or water A2, through distilling method together with oxidizing agent at its final purifying stage, or through the method to be able to get the same quality as this.

3.2.2 Test methods

(1) Matters in common

- (1.1) Reagents Reagents shall follow 3.3.
- (1.2) Water The water to be used for preparation of reagents and for test shall be the water A3 or A4 unless otherwise specified.
- (1.3) Test solution and standard solution Test solution and standard solution shall be as follows besides those specified in JIS K 8001.

When the concentration is shown in parentheses behind the nomenclature of solution at its preparation, the concentration is rough value.

- (1.4) Expression of results When 2 or more test methods are stated for one test item, the employed test method shall be expressed.

(2) Conductivity ($\mu\text{S}/\text{cm}$) (25°C) Conductivity test shall follow JIS K 0552.

- (2.1) Test by continuous measurement The test by continuous measurement shall follow as follows besides those specified in 5.2 of JIS K 0552.

As for a pipe, joint, valve, or the like, wash them with sufficient flowing of sample, connect them, control flow rate and temperature, and then introduce directly into a flowing liquid-type detector.

If necessary, a thermal exchanger or thermostat can be placed before the detector.

Confirm that the variation of flow rate gives no change on temperature and conductivity.

- (2.2) Test by intermittent measurement The sample taken or the sample for test of water A4 (don't use for tests of water A2 to A4) contained in the container shall be tested immediately after sampling according to 5.1 of JIS K 0552 besides the items stated in the following.

- (2.2.1) Sample container Sample container shall be made of hard glass, polyethylene, or polypropylene.
- (2.2.2) Washing of sample container The washing of sample container shall follow 3.7. In this case, carry out the following washing instead of rinsing.

Place water A1 into a sample container by a quarter of its capacity, and shake violently for 30 s. After repeating this procedure five times, fill it with water A3 or the water having equivalent quality to the water to be tested, let it stand for 16 h, discard the water, again fill it with water A3 or the water having equivalent quality to the water to be tested, keep it until sampling, and place it on a clean bench or in clean room, or at a place with cleanliness equivalent or superior to this.

- (2.2.3) Sampling Sampling shall be carried out as follows, so as to avoid the contamination from skin, with the hands with operational gloves, which has been washed with the water to be tested or the water having equivalent or superior quality to the water, on a clean bench or in clean room, or at a place with cleanliness equivalent or superior to this.

- (a) Attach a soft synthetic resin-made sample-introducing pipe, having washed with water A1 sufficiently, to the exit pipe of a water purifying apparatus.
- (b) Discard water in the sample container, then fill it with the water to be tested, and let it stand for 16 h.
- (c) After making the tip of the sample-introducing pipe touch the bottom of the sample container, overflow the sample by five times capacity of the sample container, and then fill it with continuously flowing sample to prepare the sample for measurement.
- (d) Remove the sample-introducing pipe.

After removing, the sample-introducing pipe shall be stoppered with a cleaned stopper and stored, in a clean place.

- (3) Organic carbon (TOC) The test of organic carbon (TOC) shall be carried out as follows besides description in JIS K 0551.

- (3.1) Test by TOC automatic measuring device Introduce the water from a water purifying apparatus into a TOC automatic measuring device according to 4.1.1, and test the water according to 5.1 or 5.3 of JIS K 0551.

The water for the preparation of working curve in this test shall be the one specified in 5.1 (2) (a) of JIS K 0551 or the water with quality equivalent to this.

- (3.2) Test by TOC analyzer

- (3.2.1) Sample container Sample container shall be airtight and made of hard glass or ethylene tetrafluoride resin.

- (3.2.2) Washing of sample container The washing of sample containers shall be carried out as follows.

- (a) Wash with nitric acid (0.2 mol/l) [Prepare with nitric acid specified in JIS K 8541 (Higher quality nitric acid is also available.) and water A1], and then wash it with water A1 sufficiently.
- (b) Place water A1 into a sample container by about a quarter of its capacity, and shake violently for about 30 s for washing. Repeat this procedure five times.
- (c) Fill the container with water A3 (or the water the same as the water to be tested), and discard the water after standing for 16 h or longer.
- (d) Fill the container with the water the same in (c), and let it stand until sampling.

- (3.2.3) Sampling Sampling shall be carried out as follows.

- (a) Discard the water in the sample container, and then carry out the operations stated in (3.2.2) (b) with the water to be tested.
- (b) Take the sample in the sample container according to (2.2.3). In this case, the sample which is to be tested immediately after purifying shall be taken from the outlet water of the water purifying apparatus.

Remarks: When water purifying apparatus has a large throughput, the following sampling method is also valid.

Attach a sampling valve at the outlet pipe of water purifying apparatus, attach a sample-introducing pipe (for instance, made of soft plastic) at the tip of a sampling valve, and then flow the sample at the rate of about 5 l/min for about 5 min. When the path from main pipe to sampling valve is long, flow out the staying water from the main pipe to the valve completely, and then flow successively for about another 5 min.

When the full open of a sampling valve does not give the flow rate of about 5 l/min, flow out about 25 l (It is advisable to flow constantly from the sampling valve at the rate of about 1 l/min). Successively, flow the water for about 30 min at the rate of about 1 l/min, place water to be tested into a sample container which has been treated as shown in (3.2.2) (a) to (d) by about a quarter of its capacity, and shake it violently for washing (repeat this five times). After making the tip of the sample-introducing pipe touch the bottom of the sample container, overflow the sample by five times capacity of the sample container, and pull out the introducing pipe. Stopper it closely with a fully washed stopper.

(3.2.4) Test Test shall be carried out as follows.

(a) Carry out the test according to 5.2 or 5.4 of JIS K 0551.

The water for the preparation of working curve in this test shall be the one specified in 5.1 (2) (a) of JIS K 0551 or the water with quality equivalent to this.

(4) Zinc The test for zinc shall follow JIS K 0553.

(4.1) Sample container Sample container shall be airtight and made of quartz glass, polyethylene, or polypropylene.

(4.2) Washing of sample container The washing of sample container shall follow 3.7.

(4.3) Sampling Sampling shall follow (2.2.3). In this case, the sample which is to be tested immediately after purifying shall be taken from the outlet water of the water purifying apparatus.

(4.4) Test Test shall be carried out as follows.

(a) Carry out the test according to 5.7 of JIS K 0553.

Remarks: When testing metal element, it is crucially important to pay attention to so that sample may not be contaminated with sampling operation. That is, give care to the material, shape, and dimensions of used implements, washing method, impurities contained in reagents, and dust floating in test surroundings, and so on. It is also necessary that the metal element has as little contact as possible with atmospheric air during the operation.

(5) Silica The test for silica shall follow JIS K 0555.

(5.1) Sample container Sample container shall be airtight and made of synthetic resin such as polyethylene or polypropylene.

(5.2) Washing of sample container Follow 3.7.

(5.3) Sampling Sampling shall follow (2.2.3). In this case, the sample which is to be tested immediately after purifying shall be taken from the outlet water of the water purifying apparatus.

Remarks: Follow the Remarks in (3.2.3).

(5.4) Test Test shall be carried out as follows.

(a) Carry out the test according to JIS K 0555.

(6) Chloride ion The test for chloride ion shall follow JIS K 0556.

(6.1) Sample container Sample container shall be airtight and made of hard glass, polyethylene, or polypropylene.

(6.2) Washing of sample container Follow 3.7.

(6.3) Sampling Sampling shall follow (2.2.3). In this case, the sample which is to be tested immediately after purifying shall be taken from the outlet water of the water purifying apparatus.

Remarks: Follow the Remarks in (3.2.3).

(6.4) Test Test shall be carried out as follows.

(a) Carry out the test⁽¹²⁾ according to 5.1 prescribed in JIS K 0556.

Note ⁽¹²⁾ Instead of the ion chromatograph specified in JIS K 0556, the ion chromatograph capable of determining 1 µgCl⁻/l may be used.

(7) Sulfate ion The test for sulfate ion shall follow JIS K 0556.

(7.1) Sample container Sample container shall follow (6.1).

(7.2) Washing of sample container The washing of sample container shall follow 3.7.

(7.3) Sampling Sampling shall follow (2.2.3). In this case, the sample which is to be tested immediately after purifying shall be taken from the outlet water of the water purifying apparatus.

Remarks: Follow the Remarks in (3.2.3).

(7.4) Test Test shall be carried out as follows.

(a) Carry out the test⁽¹³⁾ according to 5.6 prescribed in JIS K 0556.

Note ⁽¹³⁾ Instead of the ion chromatograph specified in JIS K 0556, the ion chromatograph capable of determining 2 µgSO₄²⁻/l may be used.

(8) Test by potassium permanganate Acidify water by sulfuric acid, add specified amount of 5 mmol/l potassium permanganate solution as an oxidizing agent, maintain it in a boiling water bath for specified time to make them react, find the amount of potassium permanganate consumed for the reaction, and express the volume (ml) of 5 mmol/l potassium permanganate solution. Carry out this test immediately after sampling.

(8.1) Sample container Sample container shall follow (6.1).

(8.2) Washing of sample container The washing of sample container shall follow 3.7.

(8.3) Sampling Sampling shall follow (2.2.3)

Remarks: Follow the Remarks in (3.2.3).

(8.4) Test Test shall be carried out as follows.

(8.4.1) Water and reagent solution Water and reagent solution shall be as follows.

- (a) Water Distill⁽¹⁴⁾ water A2 or A3.
- (b) Sulfuric acid (1 + 2) Add 50 ml of sulfuric acid specified in JIS K 8951 gently into 100 ml of water with stirring, and add potassium permanganate solution (3 g/l) until the solution gets faint red color.
- (c) Silver nitrate solution (200 g/l) Weigh 20 g of silver nitrate specified in JIS K 8550 in a 100 ml measuring flask, add 20 ml of water to dissolve it, dilute it with water up to the marked line, shake it, and store it in a brown colored glass bottle.
- (d) 5 mmol/l potassium permanganate solution Take 250 ml of 20 mmol/l potassium permanganate solution specified in 4.5 (7) of JIS K 8001 into a 1000 ml measuring flask using a 250 ml transfer pipet, and dilute with water up to the marked line, followed by shaking.

Standardization shall be carried out as follows: heat sodium oxalate specified in JIS K 8005 at 200°C for about 1 h, and let it cool in a desiccator. Weigh 0.42 g to the nearest 1 mg, dissolve in 20 ml of water, transfer it in a 250 ml measuring flask, and add water up to the marked line to shake well. Take 25 ml of this solution using a 25 ml transfer pipet into a 300 ml Erlenmeyer flask, dilute with water to make about 100 ml, and add 10 ml of sulfuric acid (1 + 2). Make the temperature of the liquid 25°C to 30°C, add rapidly 22 ml of prepared 5 mmol/l potassium permanganate solution using a 25 ml buret, and let it stand until red color disappears.

After decoloring, heat it at 55°C to 60°C, and titrate it with prepared 5 mmol/l potassium permanganate solution. Make it an end point when faint red color lasts for about 30 s.

Calculate the factor (*f*) of 5 mmol/l potassium permanganate solution according to the following formula.

$$f = a \times \frac{25}{250} \times \frac{b}{100} \times \frac{1}{x \times 0.001675}$$

where, a : amount of sodium oxalate (g)

b : purity of sodium oxalate (%)

x : 5 mmol/l potassium permanganate solution
needed for titration (ml)

0.001675 : amount of sodium oxalate equivalent to 1 ml of
5 mmol/l potassium permanganate solution (g)

- (e) Sodium oxalate solution (12.5 mmol/l) Weigh 1.8 g of sodium oxalate specified in JIS K 8528 to the nearest 0.1 mg, dissolve it in 200 ml of water, transfer it in a 1000 ml measuring flask, and dilute with water up to the marked line, followed by shaking.

Note ⁽¹⁴⁾ First, color by adding potassium permanganate solution (3 g/l) (This coloring shall last until after distillation.), add sulfuric acid (1 + 2) prepared in (b) to acidify slightly, and then distill it.

(8.4.2) Implement Implement shall be as follows.

- (a) Water bath The water bath shall have enough heat capacity and heating ability to keep boiling condition continuously after putting the sample in it.

(8.4.3) Operations Operations shall be as follows.

- (a) Take 100 ml of sample into a 300 ml Erlenmeyer flask using a 100 ml transfer pipet, and add 5 ml of silver nitrate solution (200 g/l) and 10 ml of sulfuric acid (1 + 2).
- (b) Add 10 ml of 5 mmol/l potassium permanganate solution using a 10 ml transfer pipet, agitate it, and immediately place it in a boiling water bath to heat for 30 min. In this case, make the liquid surface in the flask stay under the water surface in the bath, and keep the flask not to touch the bath bottom.
- (c) Take 10 ml of sodium oxalate solution (12.5 mmol/l) using a 10 ml transfer pipet, and put it in the 5 mmol/l potassium permanganate solution having heated for 30 min at (b), followed by well shaking to react them completely.
- (d) While keeping the solution at 55°C to 60°C, titrate it with 5 mmol/l potassium permanganate solution. Make it an end point when faint red color lasts for about 30 s.
- (e) Separately, carry out contrast test as follows: Take 100 ml of water [water used in (a)] into a 300 Erlenmeyer flask using a 100 ml transfer pipet, and add 5 ml of silver nitrate solution (200 g/l), 10 ml of sulfuric acid (1 + 2), and 10 ml of 5 mmol/l potassium permanganate solution using respectively a 5 ml and a 10 ml transfer pipet, followed by shaking.

- (f) Take 10 ml of sodium oxalate solution (12.5 mmol/l) using a 10 ml transfer pipet, add it into the mixed solution, shake them, and titrate it with 5 mmol/l potassium permanganate solution while keeping it at 55°C to 60°C. Make it an end point when faint red color lasts for about 30 s.
- (g) Calculate the volume of potassium permanganate consumed per 100 ml sample using the ml volume of needed 5 mmol/l potassium permanganate solution according to the following formula.

$$M = (a - b) \times f$$

where, M : consumed volume of 5 mmol/l potassium permanganate solution (ml)
 a : volume of 5 mmol/l potassium permanganate solution needed at titration of (d) (ml)
 b : volume of 5 mmol/l potassium permanganate solution needed at titration of (f) (ml)
 f : factor of 5 mmol/l potassium permanganate solution

3.3 Reagents Reagents shall be the guaranteed reagents with JIS mark, or the reagents equivalent to or superior to this.

If there is no reagents with JIS mark, use those specified in 4. or in a respective standard.

3.4 Reference material and reference solution Reference material and reference solution shall be specified in 4. or in a respective standard.

3.5 Implements Implements shall be as follows besides those specified in a respective standard.

- (1) **Glassware** Glassware shall be of hard glass first-class.

Be careful about components of glass like sodium, potassium, boron, silicon, aluminum, and others, because they may dissolve out from glass.

The ground joints of glassware are easily dirtied, so the glassware with transparent ground joints shall be used.

- (2) **Quartz implements** The quartz implements shall be made of transparent quartz, and, if equipped with ground joints, the ground joint parts shall be of transparent grinding because they are easily dirtied.
- (3) **Synthetic-resin implements** Synthetic-resin implements shall be made of ethylene tetrafluoride resin⁽¹⁵⁾, polyethylene resin⁽¹⁶⁾, and polypropylene resin⁽¹⁷⁾ which are not added with coloring agent nor filler, etc., and acrylic resin⁽¹⁸⁾ not added with flame retardant.

Notes ⁽¹⁵⁾ Heavy metals as iron, nickel, copper, zinc, and the like may slightly dissolve out.

Because there may be alien matters involved in forming, they should be observed depending on employing purpose.

- (¹⁶) Heavy metals as iron, chromium, titanium, and the like may slightly dissolve out from high density polyethylene-made implements, so they shall not be used for the test using high concentrated nitric acid.
- (¹⁷) Heavy metals as iron, titanium, and the like may slightly dissolve out, so they shall not be used for the test using high concentrated nitric acid.
- (¹⁸) Don't use this implement for the test using hydrochloric acid.
- (4) Platinum implements Platinum implements shall be those specified in JIS H 6201, JIS H 6202, and JIS H 6203. As it contains alloy components or impurities like palladium, rhodium, ruthenium, iridium, gold, silver, copper, iron, and the like, it shall be confirmed to give no harm for test before testing.

3.6 Container to store test solution The container to store test solution shall protect the sample by interrupting or absorbing from such outer effects as alien matter, humidity, ambient air, light, and so on, and its shape and material shall be as follows.

- (1) Materials The materials of storing container for test solution shall be as follows besides those specified in a respective standard.

- (a) Glass Glass shall be of hard glass first-class. For the purpose of light-shielding, the colored glass with manganese, iron, and so on shall be used. In special cases, such as bolosilicate low-alkali glass (heat-resisting glass) or quartz glass can be used.

Remarks: Glassware are superior in chemical resistance and transparency, but on the other hand are inferior in easy breakableness and in low resistance against strong alkali and fluoride.

- (b) Synthetic resin Such resins as ethylene tetrafluoride(¹⁹), polyethylene(²⁰), polypropylene(²¹), and acrylic(²²) and the like shall be used.

Notes (¹⁹) This resin has resistance against any of acid, alkali, and organic solvent, and is the best in chemical resistance of all synthetic resins.

- (²⁰) This resin is not suitable for the sample containing strong acid or organic solvent.

Because of its gas permeability, this is not suitable for handling the material easily oxidizable or hygroscopic.

- (²¹) This has superior resistance against chemicals except the special group of chemicals, and superior resistance against heat as well. Similar to polyethylene, however, this has gas permeability.

- (²²) The chemical resistance of this resin is inferior to other resins.

Remarks: Comparing with glassware, synthetic resin implements have advantages of light weight and unbreakableness, but they lack transparency and hardness which likely cause the inclusion of alien matter owing to chipping of container wall, and furthermore there is a possibility of dissolving plasticizer or antistatic agent.

There is a variation on chemical resistance and permeability between them, therefore it is important to use them properly according to the purpose.

- (2) Kind of containers The kind of containers shall be as follows.

- (a) Vial bottle Vial bottles are made of hard glass first-class, which can completely cut off the invasion of gas or microorganisms while keeping at ordinary condition, and is used under airtight condition with both a chemical resistant rubber stopper and cap seal.

Remarks: This can be used for aseptic reservation of such as vaccine, antiserum, antibody, or the like.

- (b) Glass bottle Glass bottles are shaped containers made of glass with a stopper, and can completely cut off alien matter both solid and liquid and protect inside while keeping at ordinary condition. For the purpose of light-shielding, the bottle made of colored glass containing manganese, iron, and so on shall be used. The material of a packing shall be such as ethylene tetrafluoride resin sheet or the material nonreactive with inside, and its stopper shall be screw type made of hard polypropylene or melamine resin.

- (c) Synthetic resin bottle Synthetic resin bottles are shaped containers with a stopper which can protect inside nearly like glass bottles do.

Remarks: There is a variation on chemical resistance and permeability between materials and some may adsorb constituents of solution, therefore sometimes pretreatment is required, and it is important to use them properly according to the purpose.

- (d) Ampule Ampules are the containers which can completely cut off the invasion of gas or microorganisms while keeping at ordinary condition.

Remarks: This shall be used for the cases where ordinary bottles cannot work because of strong smoking substance, the easily oxidized substance in air, or when heat sterilizing is needed, etc.

3.7 Washing and storing of containers and implements The washing and storing of containers and implements shall be as follows besides those specified in 4. or in a respective standard, and if necessary, wash them immediately before use with the water chosen according to the purpose among the waters specified in 3.2.

When there is a necessity, such treatment as drying, sterilization, or deactivation of endotoxin or the like shall be done.

Remarks: When drying in a dryer, it shall be confirmed that the drying gives no hindrance for test because the circulation of gas may cause a sort of contamination.

- (1) Washing of glassware and quartz glass implements Wash glassware and quartz glass implements sufficiently with tap water, moreover wash them with nitric acid (1 + 1) warmed at 50°C to 60°C, and then rinse sufficiently with water chosen according to the purpose among the water A2 to water A4 specified in 3.2.

In case of new implements, they shall be washed in the following order; nonionic surface active agent and tap water, the water chosen according to the purpose among the waters specified in 3.2, and acetone.

- (2) Washing of synthetic resin implements Immerse synthetic resin implements in nitric acid (1 + 3) kept in a polyethylene-made vessel for 2 h or longer, moreover wash using ultrasonic wave for 1 h or longer, and then rinse sufficiently with water chosen according to the purpose among the waters specified in 3.2.
- (3) Washing of platinum implements Immerse platinum implements in nitric acid (1 + 3) at 80°C or higher for about 1 h, and then rinse sufficiently with water chosen according to the purpose among the waters specified in 3.2.

When the contamination caused by previous sample is found, remove the contamination by fusing of potassium disulfate specified in JIS K 8783 or potassium hydrogensulfate specified in JIS K 8972, and wash it.

- (4) Washing of sample container and stopper Wash a sample container and stopper with tap water and detergent, immerse them in nitric acid (1 + 3) for about 12 h or longer, wash them with water A1 or A2 specified in 3.2, wash with hydrochloric acid (1 + 1), and then wash them with water chosen according to the purpose among the waters specified in 3.2.

In case of endotoxin test, use the water specified in 4.3.1 (1) (a) instead of the one in 3.2.

Don't use acids for washing of an aluminum lid.

- (5) Storing When storing, dry it, stopper tightly with a stopper and store it where there is no fear of contamination.

3.8 Sterilization of water, reagent, container, implement and working clothes, and inactivation or removal of endotoxin The sterilization of water, reagent, container, implement and working clothes, and inactivation or removal of endotoxin shall be carried out as follows besides being specified in a respective standard.

- (1) Sterilization of water, reagent, container, implement, and working clothes When sterilizing water, reagent, container, implement, and working clothes, check the suitability of working operation and condition corresponding to the situation and property of target objects⁽²³⁾, choose the suitable sterilizing method among those mentioned in Table 2, and carry out sterilization by means of the methods single or combined. In case of autoclaving, follow JIS K 3605.

Note (23) When checking the suitability of working operation and condition, the index bacteria suited for respective sterilization method can be used. Use the index bacteria which matches to the sterilization method to be carried out. That means the strain of bacteria which has the most strong resistance to the sterilization, and is stable and not variable while they are being stored. The index bacteria generally employed for various sterilization shall be given in Table 3.

Table 2. Various sterilizations

Methods		Objects	Operational condition
Heating method	Flame method	Articles mainly made of glass, porcelain, or metal, etc. which are not destroyed by flame	Generally, heat in flame for several seconds.
	Dry heating method	Articles mainly made of glass, porcelain, metal, fiber; and mineral oil, fat, fatty oil, and solid reagents, etc. which are durable to dry heating	Generally, 135°C to 145°C, 3 h to 5 h 160°C to 170°C, 2 h to 4 h 180°C to 200°C, 0.5 h to 1 h
	High pressure water vapor method	Articles mainly made of glass, porcelain, metal, rubber, paper, fiber; and water, culture media, solid reagent, or liquid reagent, etc., which are durable to high pressure vapor	Generally, 115°C, 30 min 121°C, 20 min 126°C, 15 min
	Flowing water vapor method	Articles mainly made of glass, porcelain, metal, rubber, fiber; and water, culture media, solid reagent, or liquid reagent, etc., which have a chance to be deteriorated by dry heating or high pressure water vapor method	Generally, carry out in flowing water vapor at 100°C for 30 min to 60 min.
	Boiling method	Articles mainly made of glass, porcelain, metal, rubber, fiber; and culture media, solid reagent, or liquid reagent, etc., which have a chance to be deteriorated by dry heating or high pressure water vapor method	Generally, immerse in boiling water and boil for 15 min or longer.
	Inter-mittent method	Articles mainly made of rubber; and culture media, solid reagent, or liquid reagent, etc., which have a chance to be deteriorated by dry heating or high pressure water vapor method	Generally, heat repeatedly 3 times to 5 times, once a day, for 30 min to 60 min for one trial, in water or flowing water vapor at 80°C to 100°C. During intermittent period, maintain them at the temperature of 20°C or higher suitable for the growth of bacteria.
Filtration method		Substances mainly as, gas, water, culture media unstable to heat, solid reagent, or liquid reagent, etc., which have a chance to be deteriorated by heating method	Generally, use membrane filter or porcelain filter, etc.

Table 2. (Continued)

Methods		Objects	Operational condition
Radiation method	Radiation method	Articles mainly made of glass, porcelain, metal, rubber, plastics, fiber, etc., which are durable to radiation	Generally, use radiation source containing ^{60}Co , ^{137}Cs , or the like.
	Ultra-violet ray method	Considerably flat surfaced articles mainly made of glass, metal, rubber, plastics, fiber; and facilities, apparatus, water, solid reagent, or liquid reagent, etc., which are durable to ultraviolet ray radiation	Generally, use ultraviolet rays with about 254 nm wavelength.
	High frequency method	Substances mainly as water, culture media, solid reagent, or liquid reagent, etc., which are durable to high frequency radiation	Generally, use high frequency wave with either 915 MHz or 2450 MHz.
Gas method		Articles mainly made of glass, porcelain, metal, rubber, plastics, fiber; and facilities, apparatus, powdery reagent, etc., which are not deteriorated by applied gas	Generally, use gas such as ethylene oxide or formaldehyde. A gas sterilizer is usually used.
Medical liquid method		Articles mainly made of glass, porcelain, metal, rubber, plastics, fiber; and fingers, aseptic box, aseptic facilities, etc., which are not deteriorated by applied medical liquid	Generally, use 0.2 g/l to 1 g/l benzalkonium chloride solution, ethanol for disinfection, phenol solution, cresol solution, formaldehyde water solution.

Table 3. Various index bacteria

Sterilizing method	Index bacteria
High pressure water vapor method	<i>Bacillus stearothermophilus</i> ATCC 7953 <i>Clostridium tetani</i> (non-toxigenic strain) NCTC 5411
Dry heating method	<i>Bacillus stearothermophilus</i> <i>Bacillus subtilis</i> var. <i>niger</i> ATCC 9372
Filtration method	<i>Serratia marcescens</i> <i>Pseudomonas diminuta</i> ATCC 19146
Radiation method	<i>Bacillus sphaericus</i> C ₁ -A <i>Bacillus pumilus</i> E-601 <i>Streptococcus faecium</i> A ₂ -1
Gas method	<i>Bacillus subtilis</i> var. <i>globigii</i> NCTC 10073 (<i>Bacillus subtilis</i> var. <i>niger</i>)

Remarks: ATCC : American Type Culture Collection
NCTC : National Collection of Type Cultures

Informative reference 1. The twelfth revised Japanese Pharmacopoeia shows various methods for sterilization and its operational condition and method. The methods for sterilization listed in Table 2 are based on the twelfth revised Japanese Pharmacopoeia.

2. The acceptability of sterilization is usually judged by aseptic test method.

The twelfth revised Japanese Pharmacopoeia specifies the aseptic test method.

3. Various index bacteria shown in Table 3 are based on the twelfth revised Japanese Pharmacopoeia.

- (2) Inactivation or removal of endotoxin For the purpose to make endotoxin inactivation, heat the implements, containers, or others, which are to be used for endotoxin test, at 250°C or higher for 90 min when they are durable to dry heating such as glass, metal, or the like, and apply radiation when they are plastics or rubber which are durable to radiation. When the amount of endotoxin is very small, such objects as water or solution, etc. which are impossible to be treated at as high as 250°C, should be heated at 121°C for 1 h using either the high-pressure steam sterilizer or small-size high-pressure steam sterilizer specified in JIS T 7322 or JIS T 7324.

When treating gas, water, filterable culture media, solid reagent dissolvable in water or solvent, liquid reagent, and so on, employ and superfiltration method if they may be deteriorated by heating. If they are not deteriorated by distillation temperature, employ superfiltration method and distillation method jointly to remove endotoxin.

Remarks: Acceptability of the method to inactivate or remove endotoxin shall be judged by any of the test methods in 4.3.

3.9 Sampling Sampling shall be as follows besides those specified in respective standard.

- (1) General sampling method For sampling, use cleaned containers and implements which have been washed and dried, and carry out under cleaned surroundings. The sample shall be possibly representative and as small amount as possible.
- (2) Sampling of unstable sample When treating sample which is easily decomposed, take care not to decompose or deteriorate it, and carry out its handling as quickly as possible.

Informative reference: In many cases, they are treated at 10°C or lower.

- (3) Aseptic sampling When aseptic sampling is needed, sampling shall be carried out as quickly as possible, using containers and implements having previously sterilized in 3.8 (1), under the condition of a bioclean room or clean circumstances that are equal to or superior to this.
- (4) Sampling for endotoxin test Use the container and implements of which endotoxin has been inactivated according to 3.8 (2).