

## 1. Reagents and Test Solutions

**Egg White** Use fresh egg white.

**Egg White TS** Weigh 10 g of egg white, add 40 ml of water, and shake.

**Eosine** See Eosine Y.

**Eosine Y**  $C_{20}H_6Br_4Na_2O_5$  (Extra grade)

**Eriochrome Black T**  $C_{20}H_{12}N_3NaO_7S$  [Eriochrome Black T (sodium 1-(1-hydroxy-2-naphthylazo)-6-nitro-2-naphthol-4-sulfonate), Guaranteed]

**Eriochrome Black T TS** Weigh 0.5 g of eriochrome black T and 4.5 g of hydroxylamine hydrochloride, and dissolve in 100 ml of ethanol. Store in a light-resistant container.

**Eriochrome Black T - Sodium Chloride Indicator** Mix 0.1 g of eriochrome black T and 10 g of sodium chloride, and triturate thoroughly to be homogeneous.

**Erythritol**  $C_4H_{10}O_4$  Erythritol occurs as white crystals or crystalline powder.

*Clarity of Solution:* Clear (1.0 g, water 20 ml).

*Melting Point:* 118 – 120 .

*Residue on Ignition:* Not more than 0.10% (2 g).

**Ethanol**  $C_2H_5OH$  [Ethanol (95) (Ethyl Alcohol (95)), Guaranteed]

**Ethanol, Absolute**  $C_2H_5OH$  [Ethanol (99.5) (Ethyl Alcohol (99.5)), Guaranteed]

**Ethanol, Aldehyde-free**  $C_2H_5OH$  Measure 1,000 ml of ethanol, add 5 ml of sulfuric acid and 20 ml of water, and distill. To 1,000 ml of the distillate, add 10 g of silver nitrate and 1 g of potassium hydroxide, equip with a reflux condenser, boil for 3 hours, and distill again.

**Ethanol, Neutralized** Measure suitable quantity of ethanol, add several drops of phenolphthalein TS, and add sodium hydroxide solution (1 : 1,250) until a light pink color develops. Prepare freshly before use.

**Ethanol-free Chloroform** See Chloroform, Ethanol-free.

**Ethanolic Potassium Hydroxide TS** See Potassium Hydroxide TS, Ethanolic.

**Ethanolic 10% Potassium Hydroxide TS** See 10% Potassium Hydroxide TS, Ethanolic.

**Ether**  $C_2H_5OC_2H_5$  (Diethylether, Guaranteed)

**Ether for Vitamin A Determination** Distill ether, and discard 10% of the initial distillate and 10% of the distillation residue. Determine the absorbance of the distillate, using redistilled water as the reference. The absorbance of the distillate is not more than 0.01 at 300 - 350 nm.

*Peroxide:* Measure 5 ml of Ether for Vitamin A Determination, and add 5 ml of iron( ) sulfate TS and 5 ml of ammonium thiocyanate solution (2 : 25). No red color develops.

**Ethyl Acetate**  $CH_3COOC_2H_5$  (Guaranteed)

**Ethyl-Ester of *N*-Benzoyl-L-Arginine Hydrochloride**  $C_{15}H_{22}N_4O_3 \cdot HCl$  Ethyl-Ester of *N*-Benzoyl-L-Arginine Hydrochloride occurs as a white crystalline powder.

*Melting point:* 128 - 133 .

*Purity:* Add water to 0.10 g of Ethyl-Ester of *N*-Benzoyl-L-Arginine Hydrochloride, and dissolve. Add water to make exactly 10 ml, and use this solution as the test solution. Use 10  $\mu$ l of the test solution, perform Thin-Layer Chromatography without a control solution, using *n*-butanol - acetic acid - water(4 : 1 : 1) as the developing solvent. Only one spot is observed. For the thin-layer plate, use a plate prepared by applying silica gel

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for thin-layer chromatography dried at 110 for 1 hour. Stop the development when the solvent front rises about 10 cm above the original line, air-dry, and allow to stand in the iodine vapor for 30 seconds.

**Ethyl Formate**  $\text{HCOOC}_2\text{H}_5$  Ethyl Formate occurs as a colorless, transparent liquid having a characteristic odor.

*Contents:* Ethyl Formate contains not less than 97.0% of  $\text{HCOOC}_2\text{H}_5$ .

*Refractive index:*  $n_D^{20} = 1.3595 - 1.3601$

*Specific gravity:*  $d_4^{20} = 0.915 - 0.924$

*Boiling point:* 53 - 54

*Assay:* Weigh accurately about 5.0 g of Ethyl Formate, and proceed as directed under Ester Value and Acid Value in Flavoring Substances Tests. Calculate the content by the following formula:

Content of Ethyl Formate ( $\text{HCOOC}_2\text{H}_5$ )

$$= \frac{\text{Saponification Value} - \text{Acid Value}}{561.1} \times 74.08 (\%).$$

**Ethylene Glycol**  $\text{HOCH}_2\text{CH}_2\text{OH}$  [Ethylene Glycol (Glycol), Guaranteed]

**Ethylene Glycol for Water Determination** Distill ethylene glycol, and use the distillate at 195 - 198 as Ethylene Glycol for Water Determination. The water content in 1 ml of it is not more than 1.0 mg.

**Ethylene Glycol Monomethyl Ether**  $\text{HOCH}_2\text{CH}_2\text{OCH}_3$  [2-Methoxyethanol (Ethylene Glycol Monomethyl Ether), Guaranteed]

**N-Ethylmaleimide**  $\text{C}_4\text{H}_2\text{O}_2\text{NC}_2\text{H}_5$  N-Ethylmaleimide occurs as white crystals. It is very soluble in ethanol and ether. N-Ethylmaleimide solution (1 10,000) exhibits an absorbance maximum at 298 - 302 nm.

*Melting Point:* 44.0 - 46.0 .

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### Fehling's TS

*Copper Solution:* Weigh 34.66 g of fine cupric sulfate crystals, and dissolve in water to make 500 ml. Store in a bottle with a ground-glass stopper, almost filled.

*Alkaline Tartrate Solution:* Weigh 173 g of potassium sodium tartrate and 50 g of sodium hydroxide, mix, and dissolve in water to make 500 ml. Store in a container with a rubber stopper.

Mix equal volumes of both solutions before use.

**Ferric Ammonium Sulfate** See Iron( ) Ammonium Sulfate.

**Ferric Ammonium Sulfate TS** Weigh 14 g of iron( ) ammonium sulfate, add 100 ml of water, and dissolve by shaking well. Filter, and add 10 ml of sulfuric acid. Store in a brown bottle.

**Ferric Ammonium Sulfate - Hydrochloric Acid TS** Freshly prepare before use.

**Ferric Ammonium Sulfate - Sulfuric Acid TS** Weigh 15 g of iron( ) ammonium sulfate, and dissolve in 90 ml of water. Filter, and add 10 ml of diluted sulfuric acid (1 : 35).

**Ferric Chloride** See Iron( ) Chloride

**Ferric Chloride - Hydrochloric Acid TS** Weigh 5 g of iron( ) chloride, and dissolve in 5 ml of hydrochloric acid and water to make 100 ml.

**Ferric Sulfate** See Iron( ) Sulfate.

**Ferric Sulfate TS** Weigh 50 g of iron( ) sulfate, add about 500 ml of water, and shake well. Add 200 ml of sulfuric acid, dissolve by shaking well, and add water to make 1,000 ml.

**Ferrous Ammonium Sulfate** See Iron( ) Ammonium Sulfate.

**Ferrous Sulfate** See Iron( ) Sulfate.

**Ferrous Sulfate TS** Weigh 8 g of iron( ) sulfate, and dissolve in 100 ml of freshly boiled and cooled water. Prepare freshly before use.

**Ferrous Sulfate TS, Acidic** Measure 7.5 ml of sulfuric acid and transfer into 100 ml of water. Dissolve about 80 g of ferrous sulfate in the previously prepared diluted sulfuric acid, while heating. Measure 7.5 ml of nitric acid, transfer into 20 ml of water, and warm. Add the former ferrous sulfate solution into the latter nitric solution. Concentrate the mixed solution until the color of solution changes black to red, evolving red vapor. Add several drops of nitric acid until the ferrous reaction no longer occurs, and warm again. After cool, add water to this concentrated solution to make 110 ml. Prepare freshly before use.

**Ferrous Sulfide** See Iron( ) Sulfide.

**Folin's TS** Weigh 20 g of sodium tungstate and 5 g of sodium molybdate, transfer into a 300-ml flask, and add about 140 ml of water, 10 ml of diluted phosphoric acid (17 : 20), and 20 ml of hydrochloric acid. Equip the flask with a reflux condenser by ground-glass joints, and boil slowly for 10 hours. Add 30 g of lithium sulfate and 10 ml of water, add a very small amount of bromine until the dark green color of the solution changes to yellow, and boil for 15 minutes without a condenser to expel the excess bromine. Cool, add water to make 200 ml, and filter through a glass filter. Store, stoppered tightly.

**Formalin** HCHO [Formaldehyde Solution (Formalin), Guaranteed]

**Formalin - Sulfuric Acid TS** Measure 0.2 ml of formalin, and mix with 10 ml

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sulfuric acid. Prepare freshly before use.

**Formic Acid**  $\text{HCOOH}$  (Guaranteed)

**Formic Acid Buffer (pH 2.5)** Measure 4 ml of formic acid, and add 90 ml of water.

Adjust the pH to 2.5 with aqueous ammonia, and add water to make 1,000 ml.

**Fructose**  $\text{C}_6\text{H}_{12}\text{O}_6$  Use fructose specified under the Japanese Pharmacopoeia.

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**Galactitol**  $C_6H_{14}O_6$  Galactitol occurs as white crystals or a crystalline powder.

*Clarity of solution:* clear (1.0 g, water 30 ml)

*Melting point:* 188 - 189 .

*Water:* Not more than 0.5 % (1.0 g, direct titration).

*Residue on Ignition:* not more than 0.10 % (2 g).

**Gallic Acid**  $C_7H_6O_5 \cdot H_2O$  (Guaranteed)

**Gelatin** Use Gelatin specified under the Japanese Pharmacopoeia.

**Gelatin TS** Dissolve gently 1 g of gelatin in water on heating, filter if necessary. Prepare freshly before use.

**Gelatin Origin Peptone** See Peptone, Gelatin Origin.

**Gel-form Strongly Acidic Cation-exchange Resin** Use gel-form strongly acidic cation-exchange resin of high quality prepared for Liquid Chromatography.

**Gel-form Sulfonated Polystyrene** Use gel-form sulfonated polystyrene of high quality prepared for Liquid Chromatography.

**General Bouillon** See Bouillon, General.

**Girard Reagent P**  $C_7H_{10}N_3OCl$  Girard Reagent P occurs as a white to light yellow-orange powder having a slight characteristic odor. It is freely soluble in water, sparingly soluble in methanol and practically insoluble in ethanol.

*Content:* Girard Reagent P contains not less than 95.0 % of acetohydrazide chloropyridinium ( $C_7H_{10}N_3OCl$ ).

*Melting point:* 200 - 203

*Assay:* Dry Girard Reagent P at 105 to constant weight, weigh accurately about 0.3 g of this dried Girard Reagent P, add 50 ml of water and dissolve. Add 3 ml of diluted nitric acid (1 : 3), titrate with 0.1 mol/l silver nitrate. The end point is confirmed by using a potentiometer. Perform a blank test in the same manner, make any necessary correction.

1 ml of 0.1 mol/l silver nitrate = 18.763 mg of  $C_7H_{10}N_3OCl$ .

**Glucose**  $C_6H_{12}O_6$  Use Glucose specified under the Japanese Pharmacopoeia.

**Glycerol**  $CH_2(OH)CH(OH)CH_2OH$  (Guaranteed)