

Turpentine Oil

Oleum Terebinthinae

テレピン油

Turpentine Oil is the essential oil distilled with steam from the wood or balsam of *Pinus* species (*Pinaceae*).

Description Turpentine Oil is a clear, colorless to pale yellow liquid. It has a characteristic odor and a pungent, bitter taste.

Turpentine Oil (1 mL) is miscible with 5 mL of ethanol (95) and this solution is neutral.

Refractive index n_D^{20} : 1.465 – 1.478

Specific gravity d_{20}^{20} : 0.860 – 0.875

Purity (1) Foreign matter—Turpentine Oil has no offensive odor. Shake 5 mL of Turpentine Oil with 5 mL of a solution of potassium hydroxide (1 in 6): the aqueous layer does not show a yellow-brown to dark brown color.

(2) Hydrochloric acid-coloring substances—Shake 5 mL of Turpentine Oil with 5 mL of hydrochloric acid, and allow to stand for 5 minutes: the hydrochloric acid layer is light yellow and not brown in color.

(3) Mineral oil—Place 5 mL of Turpentine Oil in a Cassia flask, cool to a temperature not exceeding 15°C, add dropwise 25 mL of fuming sulfuric acid while shaking, warm between 60°C and 65°C for 10 minutes, and add sulfuric acid to raise the lower level of the oily layer to the graduated portion of the neck: not more than 0.1 mL of oil separates.

Distilling range 150 – 170°C, not less than 90 vol%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Urokinase

ウロキナーゼ

[9010-53-1]

Urokinase is an enzyme, obtained from human urine, that activates plasminogen, and has the molecular mass of about 54,000. It is a solution using a suitable buffer solution as the solvent.

It contains not less than 60,000 Units per mL, and not less than 120,000 Units per mg of protein.

Description Urokinase is a clear and colorless liquid.

The pH is between 5.5 and 7.5.

Identification (1) Dissolve 0.07 g of fibrinogen in 10 mL of phosphate buffer solution, pH 7.4. To this solution add 1 mL of a solution of thrombin containing 10 Units per mL in isotonic sodium chloride solution, mix, place in a Petri dish about 90 mm in inside diameter, and keep horizontally until the solution is coagulated. On the surface drop 10 μ L of a solution of Urokinase containing 100 Units per mL in gelatin-tris buffer solution, and stand for overnight: lysis circle is ap-

peared.

(2) Dissolve 1.0 g of Powdered Agar in 100 mL of boric acid-sodium hydroxide buffer solution, pH 8.4, by warming, and pour the solution into a Petri dish until the height come to about 2 mm. After cooling, make two wells of 2.5 mm in diameter with the space of 6 mm. To each well place separately 10 μ L of a solution of Urokinase containing 30,000 Units per mL in isotonic sodium chloride solution and 10 μ L of anti-urokinase serum, and stand for overnight: a clear precipitin line is appeared.

Purity (1) Heavy metals—Proceed with 2.0 mL of Urokinase according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Blood group substances—Dilute Urokinase with isotonic sodium chloride solution so that each mL of the solution contains 12,000 Units, and use this solution as the sample solution. To anti-A type antibody for blood typing add isotonic sodium chloride solution to dilute each 32, 64, 128, 256, 512 and 1024 times, place separately 25 μ L each of these solutions in six wells on the first and second lane of a V-shaped 96-wells microplate. Next, add 25 μ L of the sample solution into the six wells on the first lane and 25 μ L of isotonic sodium chloride solution into the six wells of the second lane, mix, and allow to stand for 30 min. To each well add 50 μ L of A-type erythrocyte suspension, mix, allow to stand for 2 hours, and compare the agglutination of erythrocyte in both lanes: dilution factor of anti-A type antibody of the wells which show the agglutination is equal in both lanes.

Perform the same test by using anti-B type antibody for blood typing and B-type erythrocyte suspension.

Abnormal toxicity Dilute Urokinase with isotonic sodium chloride solution so that each mL of the solution contains 12,000 Units, and use this solution as the sample solution. Inject 5.0 mL of the sample solution into the peritoneal cavity of each of 2 or more of well-nourished, healthy guinea pigs weighing about 350 g, and inject 0.5 mL of the sample solution into the peritoneal cavity of each of 2 or more of well-nourished, healthy mice aged about 5 weeks. Observe the conditions of the animals for more than 7 days: all the animals exhibit no abnormalities.

High molecular mass urokinase Dilute Urokinase with gelatin-phosphate buffer solution so that each mL of the solution contains 10,000 Units, and use this solution as the sample solution. Perform the test with 100 μ L of the sample solution as directed under the Liquid Chromatography according to the following conditions. Determine the peak areas of two peaks eluted closely at about 35 minutes having smaller retention time, A_a , and larger retention time, A_b , by the automatic integration method: the value, $A_a / (A_a + A_b)$, is not less than 0.85.

Operating conditions—

Apparatus: Use a pumping system for the mobile phase, a sample injection port, a column, a pumping system for the reaction reagent, a reaction coil, a reaction chamber, a spectrofluorometer and a recorder. Attach a 3-way tube to the outlet for the mobile phase of the column, connect the pumping system for the reaction reagent and the reaction coil, and join outlet of the reaction coil to the spectrofluorometer.

Detector: Spectrofluorometer (excitation wavelength: 365 nm, fluorescence wavelength: 460 nm).

Column: A stainless steel column 7.5 mm in inside di-