Isophane Insulin Human (Genetical Recombination) Injectable Aqueous Suspension

イソフェンインスリン　ヒト(遺伝子組換え)水性懸濁注射液

Isophane Insulin Human (Genetical Recombination) Injectable Aqueous Suspension is an aqueous suspension for injection.

It contains not less than 95.0% and not more than 105.0% of the labeled Insulin Unit of insulin human (genetical recombination) ($C_{257}H_{383}N_{65}O_{77}S_6$: 5807.57).

Method of preparation Prepare as directed under Injections, with Insulin Human (Genetical Recombination) and Protamine Sulfate.

Description Isophane Insulin Human (Genetical Recombination) Injectable Aqueous Suspension is a white aqueous suspension. When allowed to stand, it separates into a white precipitate and colorless supernatant liquid, and the precipitate returns to the suspension state on gentle shaking.

When it is examined microscopically, the precipitate mostly consist of fine, oblong crystals of 1 to 30 μm in major axis, and does not contain amorphous substances or large aggregates.

Identification Adjust Isophane Insulin Human (Genetical Recombination) Injectable Aqueous Suspension to pH between 2.5 and 3.0 with dilute hydrochloric acid: the precipitate dissolves, and the solution is clear and colorless.

pH Being specified separately when the drug is granted approval based on the Law.

Purity (1) Desamido substance－Perform the test with 20 μL of the sample solution obtained in the Assay as directed under Liquid Chromatography *<2.01>* according to the following conditions. Determine each peak area by the automatic integration method, and calculate the amount of them by the area percentage method: the amount of the peak, having the relative retention time of about 1.3 to human insulin, is not more than 1.5%.

Operating conditions－

Proceed as directed in the operating conditions in the Assay.

System suitability－

System performance: Proceed as directed in the system suitability in the Assay.

Test for required detectability: Pipet 1 mL of the sample solution, add 0.01 mol/L hydrochloric acid TS to make exactly 50 mL. Confirm that the peak area of human insulin obtained with 20 μL of this solution is equivalent to 1.4 to 2.6% of that obtained with 20 μL of the sample solution.

System repeatability: Dissolve Insulin Human RS in 0.01 mol/L hydrochloric acid TS so that each mL contains about 4 Insulin Units. When the test is repeated 6 times with 20 μL of this solution under the above operating conditions, the relative standard deviation of the peak area of human insulin is not more than 2.0%.

(2) Dissolved insulin human－Centrifuge Isophane Insulin Human (Genetical Recombination) Injectable Aqueous Suspension, and use the supernatant liquid as the sample solution. Separately, dissolve exactly Insulin Human RS in 0.01 mol/L hydrochloric acid TS to make a solution so that each mL contains about 1.0 Insulin Units, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography *<2.01>* according to the following conditions. Determine the peak areas, A_T and A_S, of human insulin by the automatic integration method, and calculate the amount of dissolved human insulin by the following equation: not more than 0.5 Insulin Units per mL.

Amount (mg) of dissolved insulin human (Insulin Unit /mL) $= (M_S \times F) / D \times A_T / A_S$

M_S: Amount (mg) of Insulin Human RS taken
F: Labeled Unit (Insulin Unit /mg) of Insulin Human RS
D: Volume (mL) of 0.01 mol/L hydrochloric acid TS used to dissolve Insulin Human RS

Operating conditions－

Proceed as directed in the operating conditions in the Assay.

System suitability－

System performance: When the procedure is run with 20 μL of human insulin desamido substance-containing TS under the above operating conditions, human insulin and human insulin desamido substance are eluted in this order with the resolution between these peaks being not less than 2.0, and the symmetry factor of the peak of human insulin is not more than 1.6.

System repeatability: When the test is repeated 4 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of human insulin is not more than 6.0%.

(3) High-molecular mass protein－Shake gently, take a suitable volume of Isophane Insulin Human (Genetical Recombination) Injectable Aqueous Suspension, add 4 μL of 6 mol/L hydrochloric acid TS for every mL of the solution, and mix until the solution becomes clear. Perform the test with 100 μL of this solution as directed under Liquid Chromatography *<2.01>* according to the following conditions. Determine each peak area by the automatic integration method, and calculate the amount of them by the area percentage method: the total amount of the peaks other than human insulin is not more than 2.5%.

Operating conditions－

Detector, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Purity (2) under Insulin Human (Genetical Recombination).

Column: A stainless steel column 7.8 mm in inside diameter and 30 cm in length, packed with hydrophilic silica gel for liquid chromatography.

Time span of measurement: From the retention time corresponding to the exclusion volume of the size-exclusion column to the completion of the elution of human insulin.

System suitability－

System performance: Proceed as directed in the system suitability in the Purity (2) under Insulin Human (Genetical Recombination).

Test for required detectability: Pipet 1 mL of the sample solution, and add 0.01 mol/L hydrochloric acid TS to make exactly 50 mL. Confirm that the peak area of human insulin obtained with 100 μL of this solution is equivalent to 1.4 to 2.6% of that obtained with 100 μL of the sample solution.

Extractable volume *<6.05>* It meets the requirement.

Foreign insoluble matter *<6.06>* Perform the test according to Method 1: it meets the requirement.

Sterility *<4.06>* Perform the test according to the Membrane filtration method: it meets the requirement.

Zinc content Shake gently, pipet a volume of Isophane Insulin Human (Genetical Recombination) Injectable Aqueous Suspension, equivalent to 300 Insulin Units, and add 0.01 mol/L hydrochloric acid TS to make exactly 50 mL. If necessary, further add 0.01 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the sample solution. Separately, pipet a suitable volume of Standard Zinc Solution for Atomic Absorption Spectroscopy, dilute with 0.01 mol/L hydrochloric acid TS to make three solutions containing 0.20 μg, 0.60 μg and 1.20 μg of zinc (Zn: 65.38) in each mL, respectively, and use these solutions as the standard solutions. Perform the test with the sample solution and standard solutions as directed under Atomic Absorption Spectroscopy *<2.23>* according to the following conditions, using the 0.01 mol/L hydrochloric acid TS as the blank, and calculate the amount of zinc (Zn: 65.38) in the sample solution by using the calibration curve obtained from the absorbances of the standard solutions: 10 – 40 μg per 100 Insulin Units.

Gas: Combustible gas－Acetylene.
Supporting gas－Air.
Lamp: Zinc hollow cathode lamp.
Wavelenghth: 213.9 nm.

Assay Shake gently, pipet 10 mL of Isophane Insulin Human (Genetical Recombination) Injectable Aqueous Suspension, and add exactly 40 μL of 6 mol/L hydrochloric acid TS. Pipet 2 mL of this solution, add 0.01 mol/L hydrochloric acid TS to make exactly 5 mL, and use this solution as the sample solution. Separately, weigh accurately a suitable amount of Insulin Human RS, dissolve exactly in 0.01 mol/L hydrochloric acid TS to make a solution so that each mL contains about 40 Insulin Units, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography *<2.01>* according to the following conditions. Determine the peak areas, A_{TI} and A_{SI}, of human insulin and those, A_{TD} and A_{SD}, of the desamido substance having the relative retention time of about 1.3 to human insulin, respectively, in each solution.

Amount (Insulin Unit) of human insulin ($C_{257}H_{383}N_{65}O_{77}S_6$) in 1 mL

$$= (M_S \times F) / D \times (A_{TI}+A_{TD}) / (A_{SI}+A_{SD}) \times 1.004 \times 5 / 2$$

M_S: Amount (mg) of Insulin Human RS taken
F: Labeled Unit (Insulin Unit /mg) of Insulin Human RS
D: Volume (mL) of 0.01 mol/L hydrochloric acid TS used to dissolve Insulin Human RS

Operating conditions－

Detector: An ultraviolet absorption photometer (wavelength: 214 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40℃.

Mobile phase: A mixture of phosphoric acid-sodium sulfate buffer solution (pH 2.3) and acetonitrile for liquid chromatography (3:1). Adjust the mixing ratio of the component of the mobile phase so that the retention time of human insulin is between 10 and 17 minutes.

Flow rate: 1.0 mL per minute.

System Suitability－

System performance: When the procedure is run with 20 μL of human insulin desamido substance-containing TS under the above operating conditions, human insulin and human insulin desamido substance are eluted in this order with the resolution between these peaks being not less than 2.0, and the symmetry factor of the peak of human insulin is not more than 1.8.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of human insulin is not more than 1.6%.

Containers and storage Containers－Hermetic containers.

Storage－Light-resistant, at a temperature between 2℃ and 8℃ avoiding freezing.