Suspension) is a white suspension. When allowed to stand, it separates into a white precipitate and a colorless supernatant liquid, and it readily becomes a suspension again on gentle shaking.

When examined microscopically, most of the particles in the suspension are amorphous and have no uniform shape, and most of the dimension does not exceed 2 μm.

Identification Adjust the pH of Amorphous Insulin Zinc Injection (Aqueous Suspension) to between 2.5 and 3.5 with dilute hydrochloric acid: the particles dissolve, and the solution is clear and colorless.

pH 7.1 - 7.5

Purity Dissolved insulin—Perform the following test with a clear liquid obtained by centrifuging Amorphous Insulin Zinc Injection (Aqueous Suspension): not more than 2.5% of the labeled units is found.

Use the clear liquid of Amorphous Insulin Zinc Injection (Aqueous Suspension) as the sample solution. Prepare the standard solution having a concentration of 2.5% of the labeled units of Insulin Zinc Injection (Aqueous Suspension) by proceeding as directed in the Assay (iv) under Insulin Injection. Divide healthy rabbits weighing more than 1.8 kg, fasted for not less than 14 hours before injection, into 2 equal groups of not less than 3. Inject subcutaneously an amount of the standard solution or the sample solution equivalent to 0.3 units per kg of body mass to the animals of each group. Collect blood before and 1 hour and 2.5 hours after injection, then proceed as directed in the Assay (viii) under Insulin Injection, and calculate the ratio of the average blood sugar level of 1 hour and 2.5 hours after to that of before injection of each animal: the mean value for the group injected the sample solution is not less than that for the group injected the standard solution.

Nitrogen content Perform the test as directed under the Nitrogen Determination: the amount of nitrogen (N: 14.01) is not less than 0.50 mg and not more than 0.64 mg for each labeled 100 Units.

Assay **(1)** Insulin—Proceed as directed in the Assay under Insulin Injection with the clear liquid obtained from Amorphous Insulin Zinc Injection (Aqueous Suspension) by adjusting the pH to about 2.5 with diluted hydrochloric acid (1 in 100).

(2) Zinc—Measure exactly a volume of Amorphous Insulin Zinc Injection (Aqueous Suspension), equivalent to about 200 Units according to the labeled units, add 1 mL of 0.1 mol/L hydrochloric acid TS and sufficient water to make exactly 200 mL, then dilute with water to contain 0.6 to 1.0 μg of zinc (Zn: 65.39) per mL, and use this solution as the sample solution. Separately, pipet a volume of Standard Zinc Solution for atomic absorption spectrophotometry, dilute with water to contain 0.4 to 1.2 μg of zinc (Zn: 65.39) per mL, and use this solution as the standard solution. Perform the test with the sample solution and the standard solution as directed under the Atomic Absorption Spectrophotometry according to the following conditions, and determine the amount of zinc in the sample solution using the calibration curve obtained from the absorbance of the standard solution.

Gas: Combustible gas—Acetylene gas
Supporting gas—Air
Lamp: Zinc hollow-cathode lamp
Wavelength: 213.9 nm

(3) Crystalline insulin—Measure exactly a volume of Amorphous Insulin Zinc Injection (Aqueous Suspension), equivalent to about 1000 Units according to the labeled units, centrifuge, discard the supernatant liquid, suspend the residue in 5 mL of water, add 10 mL of sodium acetate-acetone TS, shake for 3 minutes, and centrifuge. Discard the supernatant liquid, and repeat the above treatment on the residue. Wash down the residue into a Kjeldahl flask with 15 mL of sulfuric acid, and perform the test as directed under the Nitrogen Determination: the amount of nitrogen (N: 14.01) is not more than 10% of the total nitrogen content. Calculate the total nitrogen content for insulin Units of the sample taken from the values of nitrogen obtained in the Nitrogen content.

Containers and storage Containers—Hermetic containers.
Storage—In a cold place, and avoid freezing.

Expiration date 24 months after preparation.

Crystalline Insulin Zinc Injection (Aqueous Suspension)

結晶性インスリン亜鉛水性懸濁注射液

Crystalline Insulin Zinc Injection (Aqueous Suspension) is an aqueous suspension for injection. It contains not less than 90% and not more than 110% of the labeled Insulin Units, and not less than 0.12 mg and not more than 0.30 mg of zinc (Zn: 65.39) for each labeled 100 Units.

Method of preparation Prepare as directed under Injections, with Insulin and Zinc Chloride. Each 100 mL of Crystalline Insulin Zinc Injection (Aqueous Suspension) contains 0.15 to 0.17 g of Sodium Acetate, 0.65 to 0.75 g of Sodium Chloride, and 0.09 to 0.11 g of Methyl Parahydroxybenzoate.

Description Crystalline Insulin Zinc Injection (Aqueous Suspension) is a white suspension. When allowed to stand, it separates into a white precipitate and a colorless supernatant liquid, and it readily becomes a suspension again on gentle shaking.

When it is examined microscopically, most part of the particles in the suspension are crystals, the dimension of which is mostly 10 to 40 μm.

Identification Adjust the pH of Crystalline Insulin Zinc Injection (Aqueous Suspension) to between 2.5 and 3.5 with dilute hydrochloric acid: the particles dissolve, and the solution is clear and colorless.

pH 7.1 - 7.5

Purity Dissolved insulin—Perform the following test with a clear liquid obtained by centrifuging Crystalline Insulin Zinc Injection (Aqueous Suspension): not more than 2.5% of the labeled units is found.

Use the clear liquid of Crystalline Insulin Zinc Injection (Aqueous Suspension) as the sample solution. Prepare the standard solution having a concentration of 2.5% of the la-

beled units of Insulin Zinc Injection (Aqueous Suspension) by proceeding as directed in the Assay (iv) under Insulin Injection. Divide healthy rabbits weighing more than 1.8 kg, fasted for not less than 14 hours before injection, into 2 equal groups of not less than 3. Inject subcutaneously an amount of the standard solution or the sample solution equivalent to 0.3 units per kg of body mass to the animals of each group. Collect blood before and 1 hour and 2.5 hours after injection, then proceed as directed in the Assay (viii) under Insulin Injection, and calculate the ratio of the average blood sugar level of 1 hour and 2.5 hours after to that of before injection of each animal: the mean value for the group injected the sample solution is not less than that for the group injected the standard solution.

Nitrogen content Perform the test as directed under the Nitrogen Determination: not less than 0.50 mg and not more than 0.64 mg of nitrogen (N: 14.01) is found for each labeled 100 Units.

Assay **(1)** Insulin—Proceed as directed in the Assay under Insulin Injection with the clear liquid obtained from Crystalline Insulin Zinc Injection (Aqueous Suspension) by adjusting the pH to about 2.5 with diluted hydrochloric acid (1 in 100).

(2) Zinc—Measure exactly a volume of Crystalline Insulin Zinc Injection (Aqueous Suspension), equivalent to about 200 Units according to the labeled units, add 1 mL of 0.1 mol/L hydrochloric acid TS and sufficient water to make exactly 200 mL, then dilute with water to contain 0.6 to 1.0 μg of zinc (Zn: 65.39) per mL, and use this solution as the sample solution. Separately, pipet a volume of Standard Zinc Solution for atomic absorption spectrophotometry, dilute with water to contain 0.4 to 1.2 μg of zinc (Zn: 65.39) per mL, and use this solution as the standard solution. Perform the test with the sample solution and the standard solution as directed under the Atomic Absorption Spectrophotometry according to the following conditions, and determine the amount of zinc in the sample solution using the calibration curve obtained from the absorbance of the standard solution.

Gas: Combustible gas—Acetylene gas
Supporting gas—Air
Lamp: Zinc hollow-cathode lamp
Wavelength: 213.9 nm

(3) Crystalline insulin—Measure accurately a volume of Crystalline Insulin Zinc Injection (Aqueous Suspension), equivalent to about 400 Units according to the labeled Units, centrifuge, discard the supernatant liquid, suspend the residue in 5 mL of water, add 10 mL of sodium acetate-acetone TS, shake for 3 minutes, and centrifuge. Discard the supernatant liquid, and repeat the above treatment on the residue. Wash down the residue into a Kjeldahl flask with 15 mL of sulfuric acid, and perform the test as directed under the Nitrogen Determination: the amount of nitrogen (N: 14.01) is not less than 85% of the total nitrogen content. Calculate the total nitrogen content for insulin Units of a sample from the values of nitrogen obtained in the Nitrogen content.

Containers and storage Containers—Hermetic containers.
Storage—In a cold place, and avoid freezing.

Expiration date 24 months after preparation.

Insulin Zinc Protamine Injection (Aqueous Suspension)

プロタミンインスリン亜鉛水性懸濁注射液

Insulin Zinc Protamine Injection (Aqueous Suspension) is an aqueous suspension for injection. It contains not less than 90% and not more than 110% of the labeled Insulin Units, and not less than 0.12 mg and not more than 0.30 mg of zinc (Zn: 65.39) for each labeled 100 Units.

Method of preparation Prepare as directed under Injections, with Insulin, Protamine Sulfate and Zinc Chloride. It contains 0.38 to 0.63 g of Dibasic Sodium Phosphate, 1.4 to 1.8 g of Concentrated Glycerin, and 0.18 to 0.22 g of Cresol or 0.22 to 0.28 g of Phenol for each 100 mL of Insulin Zinc Protamine Injection (Aqueous Suspension).

Description Insulin Zinc Protamine Injection (Aqueous Suspension) is a white suspension. When allowed to stand, it separates into a white precipitate and a colorless, supernatant liquid, and it readily becomes suspension again on gentle shaking.

When it is examined microscopically, no large particles are seen.

Identification Adjust the pH of Insulin Zinc Protamine Injection (Aqueous Suspension) to between 2.5 and 3.5 with dilute hydrochloric acid: the particles dissolve, and the solution is clear and colorless.

pH 7.0 - 7.4

Purity **(1)** Protein—Perform the test as directed under the Nitrogen Determination: not exceeding 1.25 mg of nitrogen (N: 14.01) is found for each labeled 100 Units.

(2) Dissolved insulin—Perform the following test with the clear liquid obtained by centrifuging Insulin Zinc Protamine Injection (Aqueous Suspension): not more than 2.5% of the labeled Units is found.

Use a clear liquid of Insulin Zinc Protamine Injection (Aqueous Suspension) as the sample solution, and prepare the standard solution by proceeding as directed in the Assay (iv) under Insulin Injection to adjust its concentration to 2.5% of the labeled units of Insulin Zinc Protamine Injection (Aqueous Suspension). Divide the healthy rabbits weighing not less than 1.8 kg, fasted for not less than 14 hours before injection, into 2 equal groups of not less than 3. Inject subcutaneously an amount of the standard solution or the sample solution equivalent to 0.3 units per kg of body mass. Collect blood before and 1 hour and 2.5 hours after injection, proceed as directed in the Assay (viii) under Insulin Injection, and calculate the ratios of the average blood sugar content in each rabbit measured 1 hour and 2.5 hours after injection to the content before injection: the mean value for the group injected with the sample solution is not less than the mean value for the group injected with the standard solution.

Assay **(1)** Insulin—Proceed as directed in the Assay under Injection with the clear liquid obtained by adjusting the pH to 2.5 with diluted hydrochloric acid (1 in 100).

(2) Zinc—Measure accurately a volume of Insulin Zinc