

1 Isophane Insulin Human (Genetical 2 Recombination) Injectable Aqueous Suspension

3 イソフェンインスリン ヒト(遺伝子組換え)水性懸濁注射
4 液

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

9 It contains not less than 95.0% and not more than 105.0%
10 of the labeled Insulin Unit of insulin human (genetical
11 recombination) (C₂₅₇H₃₈₃N₆₅O₇₇S₆: 5807.57).

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

15 **Description** Isophane Insulin Human (Genetical Recombina-
16 tion) Injectable Aqueous Suspension is a white aqueous suspen-
17 sion. When allowed to stand, it separates into a white precipitate
18 and colorless supernatant liquid, and the precipitate returns to the
19 suspension state on gentle shaking.

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

23 **Identification** Adjust Isophane Insulin Human (Genetical Re-
24 combination) Injectable Aqueous Suspension to pH between 2.5
25 and 3.0 with dilute hydrochloric acid: the precipitate dissolves,
26 and the solution is clear and colorless.

27 **pH** Being specified separately when the drug is granted ap-
28 proval based on the Law.

29 **Purity (1)** Desamido substance—Perform the test with 20 μL
30 of the sample solution obtained in the Assay as directed under Liq-
31 uid Chromatography <2.01> according to the following conditions.
32 Determine each peak area by the automatic integration method,
33 and calculate the amount of them by the area percentage method:
34 the amount of the peak, having the relative retention time of about
35 1.3 to human insulin, is not more than 1.5%.

36 *Operating conditions*—

37 Proceed as directed in the operating conditions in the Assay.

38 *System suitability*—

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

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

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

51 (2) Dissolved insulin human—Centrifuge Isophane Insulin
52 Human (Genetical Recombination) Injectable Aqueous Suspen-
53 sion, and use the supernatant liquid as the sample solution. Sepa-
54 rately, dissolve exactly Insulin Human RS in 0.01 mol/L hydro-
55 chloric acid TS to make a solution so that each mL contains about
56 1.0 Insulin Units, and use this solution as the standard solution.
57 Perform the test with exactly 20 μL each of the sample solution
58 and standard solution as directed under Liquid Chromatography
59 <2.01> according to the following conditions. Determine the peak
60 areas, A_T and A_S, of human insulin by the automatic integration
61 method, and calculate the amount of dissolved human insulin by
62 the following equation: not more than 0.5 Insulin Units per mL.

63 Amount (mg) of dissolved insulin human (Insulin Unit/mL) = (M_S
64 × F) / D × A_T / A_S

65 M_S: Amount (mg) of Insulin Human RS taken

66 F: Labeled Unit (Insulin Unit/mg) of Insulin Human RS

67 D: Volume (mL) of 0.01 mol/L hydrochloric acid TS used to
68 dissolve Insulin Human RS

69 *Operating conditions*—

70 Proceed as directed in the operating conditions in the Assay.

71 *System suitability*—

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

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

82 (3) High-molecular mass protein—Shake gently, take a suita-
83 ble volume of Isophane Insulin Human (Genetical Recombina-
84 tion) Injectable Aqueous Suspension, add 4 μL of 6 mol/L hydro-
85 chloric acid TS for every mL of the solution, and mix until the
86 solution becomes clear. Perform the test with 100 μL of this solu-
87 tion as directed under Liquid Chromatography <2.01> according to
88 the following conditions. Determine each peak area by the auto-
89 matic integration method, and calculate the amount of them by the
90 area percentage method: the total amount of the peaks other than
91 human insulin is not more than 2.5%.

92 *Operating conditions*—

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

96 Column: A stainless steel column 7.8 mm in inside diameter and
97 30 cm in length, packed with hydrophilic silica gel for liquid
98 chromatography.

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

102 *System suitability*—

103 System performance: Proceed as directed in the system suitability
104 test in the Purity (2) under Insulin Human (Genetical Recombina-
105 tion).

106 Test for required detectability: Pipet 1 mL of the sample solu-
107 tion, and add 0.01 mol/L hydrochloric acid TS to make exactly 50
108 mL. Confirm that the peak area of human insulin obtained with
109 100 μL of this solution is equivalent to 1.4 to 2.6% of that obtained
110 with 100 μL of the sample solution.

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

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

114 **Sterility** <4.06> Perform the test according to the Membrane fil-
115 tration method: it meets the requirement.

116 **Zinc content** Shake gently, pipet a volume of Isophane Insulin
117 Human (Genetical Recombination) Injectable Aqueous Suspen-
118 sion, equivalent to 300 Insulin Units, and add 0.01 mol/L hydro-
119 chloric acid TS to make exactly 50 mL. If necessary, further add
120 0.01 mol/L hydrochloric acid TS to make exactly 100 mL, and use
121 this solution as the sample solution. Separately, pipet a suitable
122 volume of Standard Zinc Solution for Atomic Absorption Spec-
123 troscopy, dilute with 0.01 mol/L hydrochloric acid TS to make
124 three solutions containing 0.20 μg , 0.60 μg and 1.20 μg of zinc
125 (Zn: 65.38) in each mL, respectively, and use these solutions as
126 the standard solutions. Perform the test with the sample solution
127 and standard solutions as directed under Atomic Absorption Spec-
128 troscopy <2.23> according to the following conditions, using the
129 0.01 mol/L hydrochloric acid TS as the blank, and calculate the
130 amount of zinc (Zn: 65.38) in the sample solution by using the
131 calibration curve obtained from the absorbances of the standard
132 solutions: 10 – 40 μg per 100 Insulin Units.

133 Gas: Combustible gas – Acetylene.

134 Supporting gas – Air.

135 Lamp: Zinc hollow cathode lamp.

136 Wavelength: 213.9 nm.

137 **Assay** Shake gently, pipet 10 mL of Isophane Insulin Human
138 (Genetical Recombination) Injectable Aqueous Suspension, and
139 add exactly 40 μL of 6 mol/L hydrochloric acid TS. Pipet 2 mL of
140 this solution, add 0.01 mol/L hydrochloric acid TS to make exactly
141 5 mL, and use this solution as the sample solution. Separately,
142 weigh accurately a suitable amount of Insulin Human RS, dissolve
143 exactly in 0.01 mol/L hydrochloric acid TS to make a solution so
144 that each mL contains about 40 Insulin Units, and use this solution
145 as the standard solution. Perform the test with exactly 20 μL each
146 of the sample solution and standard solution as directed under Liq-
147 uid Chromatography <2.01> according to the following conditions.
148 Determine the peak areas, A_{TI} and A_{SI} , of human insulin and those,
149 A_{TD} and A_{SD} , of the desamido substance having the relative reten-
150 tion time of about 1.3 to human insulin, respectively, in each solu-
151 tion.

152 Amount (Insulin Unit) of human insulin ($\text{C}_{257}\text{H}_{383}\text{N}_{65}\text{O}_{77}\text{S}_6$) in 1
153 mL

$$154 = (M_S \times F) / D \times (A_{\text{TI}} + A_{\text{TD}}) / (A_{\text{SI}} + A_{\text{SD}}) \times 1.004$$

$$155 \times 5 / 2$$

156 M_S : Amount (mg) of Insulin Human RS taken

157 F : Labeled Unit (Insulin Unit /mg) of Insulin Human RS

158 D : Volume (mL) of 0.01 mol/L hydrochloric acid TS used to
159 dissolve Insulin Human RS

160 **Operating conditions** –

161 Detector: An ultraviolet absorption photometer (wavelength:
162 214 nm).

163 Column: A stainless steel column 4.6 mm in inside diameter and
164 15 cm in length, packed with octadecylsilanized silica gel for liq-
165 uid chromatography (5 μm in particle diameter).

166 Column temperature: A constant temperature of about 40°C.

167 Mobile phase: A mixture of phosphoric acid-sodium sulfate
168 buffer solution (pH 2.3) and acetonitrile for liquid chromatog-
169 raphy (3:1). Adjust the mixing ratio of the component of the mo-
170 bile phase so that the retention time of human insulin is between
171 10 and 17 minutes.

172 Flow rate: 1.0 mL per minute.

173 **System Suitability** –

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

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

184 **Containers and storage** Containers – Hermetic containers.

185 Storage – Light-resistant, at a temperature between 2°C and
186 8°C avoiding freezing.