Glucagon (Genetical Recombination)

グルカゴン(遺伝子組換え)

HSQGTFTSDY SKYLDSRRAQ DFVQWLMNT

$C_{153}H_{225}N_{43}O_{49}S$: 3482.75

[*16941-32-5*]

Glucagon (Genetical Recombination) is human Glucagon (Genetical Recombination), and is a peptide consisting of 29 amino acid residues.

It contains not less than 92.5% and not more than 105.0% of glucagon, calculated on the anhydrous basis.

Manufacture Glucagon (Genetical Recombination) is manufactured by the method that has been properly verified to be able to manufacture the drug substance having predefined biological activity. When the residual amount of host cell proteins is determined by an enzyme immunoassay as in-process tests, the amount should be not more than the reference value. In addition, Glucagon (Genetical Recombination) is purified by the method that has been verified that the residual amount of host cell DNA is not more than the reference value.

Description Glucagon (Genetical Recombination) occurs as a white lyophilized powder.

It is practically insoluble in water and in ethanol (99.5).

It is hygroscopic.

Identification (1) Dissolve 5 mg of Glucagon (Genetical Recombination) in 1 mL of 0.01 mol/L hydrochloric acid TS. To 200 μL of this solution add 800 μL of 0.1 mol/L ammonium hydrogen carbonate TS and 25 μL of enzyme TS for glucagon, react at 37℃ for 2 hours, add 120 μL of acetic acid (100) to stop the reaction, and use this solution as the sample solution. Separately, dissolve a suitable amount of Glucagon RS in 0.1 mol/L ammonium hydrogen carbonate TS so that each mL contains 1 mg of glucagon. To 1000 μL of this solution add 25 μL of enzyme TS for glucagon, react at 37℃ for 2 hours, add 120 μL of acetic acid (100) to stop the reaction, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography *<2.01>* according to the following conditions, and compare the chromatograms obtained from these solutions: both chromatograms show the similar peaks at the same retention time.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 215 nm).

Column: A stainless steel column 4 mm in inside diameter and 50 mm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 22℃.

Mobile phase A: To 0.5 mL of trifluoroacetic acid add 1000 mL of water.

Mobile phase B: To 0.5 mL of trifluoroacetic acid add 600 mL of ethanol (99.5) and 400 mL of water.

Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 － 35	100 → 53	0 → 47
35 － 45	53 → 0	47 → 100

Flow rate: 1.0 mL per minute.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the peaks 1, 2, 3, 4 and 5 are eluted in this order, and the resolution between the peak 2 and the peak 3 is not less than 1.5.

(2) Perform the test with 15 μL each of the sample solution and standard solution as directed under Liquid chromatography *<2.01>* according to the conditions described in the Assay: the retention times of the principal peaks obtained from the sample solution and the standard solution are the same.

Purity Related substances and desamide substances— Conduct this procedure at a temperature between 2℃ and 8℃. Dissolve 50 mg of Glucagon (Genetical Recombination) in 100 mL of 0.01 mol/L hydrochloric acid TS, and use this solution as the sample solution. Perform the test with 15 μL of the sample 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 amounts of them by the area percentage method: the total amount of desamide substance 1 having the relative retention time of about 1.1 to glucagon, desamide substance 2 having the relative retention time of about 1.2, desamide substance 3 having the relative retention time of about 1.3 and desamide substance 4 having the relative retention time of about 1.4 is not more than 0.8%, and the total amount of peaks other than glucagon is not more than 2.0%.

Operating conditions—

Detector, column, column temperature, mobile phases A and B, flowing of mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: For 37 minutes after injection, beginning after the solvent peak.

System suitability—

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

Test for required detectability: When the procedure is run with 15 μL of the standard solution obtained in the Assay under the above operating conditions, the peak corresponding to the desamide substance 2 is detected.

Water *<2.48>* Not more than 10% (50 mg, coulometric titration).

Assay Conduct this procedure at a temperature between 2℃ and 8℃. Weigh accurately about 50 mg of Glucagon (Genetical Recombination), dissolve in 100 mL of 0.01 mol/L hydrochloric acid TS, and use this solution as the sample solution. Separately dissolve Glucagon RS in 0.01 mol/L hydrochloric acid TS so that each mL contains about 0.5 mg of glucagon, and use this solution as the standard solution. Perform the test with exactly 15 μL each of the sample solution and standard solution as directed under Liquid Chromatography *<2.01>* according to the following conditions, and determine the peak areas, A_T and A_S, of glucagon in each solution.

$$\text{Amount (\%) of glucagon} = A_T / A_S \times C_S / C_T \times 100$$

C_S: Concentration (mg/mL) of the standard solution

C_T: Concentration (mg/mL) of the sample solution

The calculated amount (%) of glucagon is corrected by the water content to obtain the amount (%) of glucagon on the anhydrous basis.

Operating conditions—

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

Column: A stainless steel column 3 mm in inside diameter and 150 mm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μm in particle diameter).

Column temperature: A constant temperature of about 45℃.

Mobile phase A: Dissolve 16.3 g of potassium dihydrogen phosphate in 750 mL of water, adjust to pH 2.7 with phosphoric acid, add water to make 800 mL, and add 200 mL of acetonitrile for liquid chromatography.

Mobile phase B: A mixture of water and acetonitrile (3:2).

Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 — 25*	61	39
25 — 29	61 → 12	39 → 88
29 — 30	12	88
30 — 31	12 → 61	88 → 39
31 — 37	61	39

*Adjust the time for the isocratic condition so that the gradient starts after the desamide substance 4 is eluted.

Flow rate: 0.5 mL per minute.

System suitability—

System performance: Dissolve Glucagon RS in 0.01 mol/L hydrochloric acid TS to make a solution so that each mL contains 0.5 mg of glucagon. Warm this solution at 50℃ for 48 hours, and use this solution as the solution for system suitability test. When the procedure is run with 15 μL of the solution for system suitability test under the above operating conditions, four peaks corresponding to the desamide substances 1, 2, 3 and 4 eluted after the principal peak are clearly detected, the total amount of these peaks is not less than 7%, and the resolution between glucagon and the desamide substance 1 is not less than 1.5. Furthermore, when the procedure is run with 15 μL of the standard solution under the above operating conditions, the symmetry factor of the principal peak is not more than 1.8.

System repeatability: When the test is repeated 5 times with the standard solution under the above operating conditions, the relative standard deviation of the peak area of glucagon is not more than 2.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant, and not exceeding –15℃.

Add the following to 9.01 Reference Standards (1):

Glucagon RS

Add the following to 9.41 Reagents, Test Solutions:

0.1 mol/L Ammonium hydrogen carbonate TS Dissolve 7.9 g of ammonium hydrogen carbonate in 500 mL of water. Adjust to pH 10.3 with 5 mol/L sodium hydroxide TS, and add water to make 1000 mL.

α-Chymotrypsin A slightly yellowish white lyophilized powder. It contains not less than 350 U per mg of α-chymotrypsin.

Enzyme TS for glucagon Dissolve 2 mg of α-chymotrypsin in 1 mL of 0.1 mol/L ammonium hydrogen carbonate TS.