Lumbrokinase

L1491906

Storage: Room Temperature. Desiccated.

Introduction:

Earthworm Fibrinolytic Enzyme (Lumbrokinase) is a type of enzyme extracted and purified from earthworms that can dissolve fibrin. It is present in the digestive tract of earthworms. Earthworm Fibrinolytic Enzyme has a dual function: in addition to directly hydrolyzing fibrin, it can also activate plasminogen into plasmin, thereby exerting an indirect fibrin-hydrolyzing effect. After animals take Earthworm Fibrinolytic Enzyme, obvious antithrombotic and thrombolytic effects are produced.

Lumbokinase attenuates myocardial ischemia-reperfusion (I-R) injury through the activation of Sirt1 signaling, and thus enhances autophagic flux and reduces I-R-induced oxidative damage, inflammation and apoptosis.

Characteristics:

This product is a pale yellow to yellowish-brown powder; it has a fishy odor and is hygroscopic.

Identification:

1. Take an appropriate amount of this product, add water to prepare a solution containing 0.5mg per 1ml, and determine according to the spectrophotometry (Appendix IV A of the 2000 Edition of Pharmacopoeia of the People's Republic of China, Volume II); there shall be a maximum absorption at a wavelength of 278nm.
2. Take an appropriate amount of this product, add 0.9% sodium chloride solution to prepare a solution containing 10mg per 1ml as the test solution; use a No. 6 needle to take 1 drop of animal blood and place it at the bottom of a test tube. After 30 minutes, add 1ml of the test solution to the test tube, shake gently, and the blood clot shall dissolve within 60 minutes. Use 1ml of 0.9% sodium chloride solution as the blank, and perform the same operation; the blood clot shall not dissolve

Tests:

1. Acidity and Alkalinity: Take this product, add water to prepare a solution containing 1mg per 1ml, and determine according to the method (Appendix VI H of the 2000 Edition of Pharmacopoeia of the People's Republic of China, Volume II); the pH value shall be 6.0 - 8.0.
2. Clarity and Color of Solution: Take an appropriate amount of this product, add water to prepare a solution containing 1mg per 1ml; the solution shall be clear. If colored, it shall not be darker than the Yellow No. 2 Standard Color Solution (Method 1 of Appendix IX A of the 2000 Edition of Pharmacopoeia of the People's Republic of China, Volume II).

3. Loss on Drying: Take an appropriate amount of this product, use phosphorus pentoxide as a desiccant, dry it under reduced pressure at 60℃ to constant weight; the weight loss shall not exceed 5.0% (Appendix VIII L of the 2000 Edition of Pharmacopoeia of the People's Republic of China, Volume II).Determination of Specific Activity.

Titer Determination:

1. Reagents:

a) 0.01mol phosphate buffer (pH 7.8): Take 3.58g of disodium hydrogen phosphate, add water to dissolve and dilute to 1000ml as Solution A; take 0.78g of sodium dihydrogen phosphate ($NaH_2PO_4 \cdot 2H_2O$), add water to dissolve and dilute to 500ml as Solution B; mix Solutions A and B until the pH value reaches 7.8.

b) Working Solution: Mix 0.01mol/L phosphate buffer (pH 7.8) and 0.9% sodium chloride solution at a ratio of 1:17.

c) 1.5% Agarose Solution: Take 1.5g of agarose, add 100ml of the working solution, and heat to dissolve.

d) Fibrinogen Solution: Take an appropriate amount of fibrinogen, add the working solution to prepare a coagulable protein solution containing 1.5mg per 1ml.

e) Thrombin Solution: Take thrombin, add 0.9% sodium chloride solution to prepare a solution containing 1 BP unit per 1ml.

2. Preparation of Standard Solution: Take the lumbrokinase standard, add 0.9% sodium chloride solution to prepare solutions with concentrations of 10,000, 8,000, 6,000, 4,000, and 2,000 lumbrokinase units per 1ml respectively.

3. Preparation of Test Solution: Take an appropriate amount of this product, add 0.9% sodium chloride solution to dissolve and dilute to a concentration within the range of the standard curve.

4. Determination Method: Take 39ml of the fibrinogen solution, place it in a beaker, add 39ml of the 55℃ agarose solution and 3.0ml of the thrombin solution while stirring, mix immediately, and quickly pour into a plastic petri dish with a diameter of 14cm. Place it horizontally at room temperature for 1 hour, then punch holes. Precisely measure 10μl each of the lumbrokinase standard solutions of different concentrations and the test solution, spot them on the same petri dish respectively, cover the dish, and place it in a 37℃ incubator for 18 hours of reaction. After taking it out, use a caliper to measure the vertical diameters of the lysis zones. Take the logarithm of the number of units of the kinase standard as the abscissa and the logarithm of the product of the vertical diameters as the ordinate, calculate the regression equation, and substitute the logarithm of the product of the vertical diameters of the test sample into the regression equation to calculate the titer units of the test sample. Both the standard and the test sample shall be tested in duplicate, and the average value shall be used for calculation.

Protein Content:

Take about 20mg of this product, weigh it accurately, determine according to the nitrogen

determination method (Method 2 of Appendix VII D of the 2000 Edition of Pharmacopoeia of the People's Republic of China, Volume II), multiply the result by 6.25 to get the protein content in the test sample, and calculate the milligrams of protein per 1mg of the test sample.

Specific Activity:

Calculate the specific activity according to the following formula:

$$Specific\ Activity = \frac{Titer\ Units\ per\ 1mg\ of\ Test\ Sample}{Milligrams\ of\ Protein\ per\ 1mg\ of\ Test\ Sample}$$