

Alteplase

SYQVICRDEK TQMIYQQHQS WLRPVLRSNR VEYCWNSGR AQCHSVPVK
CSEPRCFNGG TCQQALYFSD FVCQCEGFA GKCEIDTRA TCYEDQGISY
RGTWSTAESG AECTNWNSSA LAQKPYSGRR PDAIRLGLGN HNYCRNPDRD
SKPWCVYFKA GKYSSEFCST PACSEGNSDC YFGNGSAYRG THSLTESGAS
CLPWNSMILI GKVYTAQNPS AQALGLGKHN YCRNPDGDAK PWCHVLKNRR
LTWEYCDVPS CSTCGLRQYS QPQFR

IKGGLFADIA SHPWAAIFA KHRRSPGERF LCGGILISSC WILSAAHCFQ
ERFPPHHLTV ILGRTRYRVP GEEQKFEVE KYIVHKEFDD DTYDNDIALL
QLKSDSSRCA QESSVVRTVC LPPADLQLPD WTECELSGYG KHEALSPFYS
ERLKEAHVRL YPSSRCTSQH LLNRTVDNM LCAGDTRSGG PQANLHDAQC
GDSGGPLVCL NDGRMTLVGI ISWGLGCGQK DVPGVYKVT NYLDWIRDNM
RP

C₂₅₆₉H₃₈₉₄N₇₄₆O₇₈₁S₄₀ 59,007.61 CAS RN®: 105857-23-6; UNII: 1RXS4UE564.

DEFINITION

Alteplase is a highly purified glycosylated serine protease with fibrin-binding properties and plasminogen-specific proteolytic activities. It is produced by recombinant DNA synthesis in mammalian cell culture. It has a biological potency of NLT 90.0% and NMT 115.0% of the potency stated on the label, the potency being 580,000 USP Alteplase Units/mg of protein. The presence of host cell DNA and host cell protein impurities in Alteplase is process specific; the limits of these impurities are determined by validated methods.

IDENTIFICATION

- A.**
Standard solution: 1.0–2.5 mg/mL of [USP Alteplase RS](#)
Sample solution: Prepare similarly to the *Standard solution*.
Analysis

Samples: *Standard solution* and *Sample solution*

To each of three test tubes transfer 1 mL of 0.5-mg/mL H-D-isoleucyl-prolyl-arginyl-p-nitroaniline dihydrochloride. Separately transfer 200 µL of the *Standard solution* and 200 µL of the *Sample solution* to two of the test tubes. To the third test tube add 200 µL of 0.2 M arginine solution that has been adjusted with phosphoric acid to a pH of 7.3 (negative control). Mix the solutions in the three test tubes, and allow to stand for 1 min.

Acceptance criteria: A yellow color is produced in the solutions from the *Standard solution* and the *Sample solution*, while no yellow color is produced in the negative control.

- B. PEPTIDE MAPPING**
Solution A: 6.9 mg/mL of monobasic sodium phosphate in water, adjusted with phosphoric acid to a pH of 2.85. Filter, and degas.
Solution B: Acetonitrile
Mobile phase: See [Table 1](#).

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
91	70	30
121	40	60
131	40	60

Dialysis solution: 480 mg/mL of urea, 44 mg/mL of tris(hydroxymethyl)aminomethane, and 0.88 mg/mL of edetic acid in water. Adjust with hydrochloric acid to a pH of 8.6.

Standard solution: Prepare a solution containing 1.0 mg/mL of [USP Alteplase RS](#) in water. Dialyze 2.0 mL of this solution into the *Dialysis solution* at room temperature for NLT 12 h. Measure the volume of the solution, and transfer it to a clean test tube. For each mL of solution in the tube, add 10 µL of 1 M dithiothreitol. Incubate at room temperature for 4 h, then add 25 µL of 1 M iodoacetic acid per mL of the solution, and incubate in the dark for 30 min. Quench the reaction by adding 50 µL of 1 M dithiothreitol per mL of the solution. Dialyze the solution against 0.1 M ammonium bicarbonate for 24 h, replacing the 0.1 M ammonium bicarbonate twice during the dialysis period. To 2.0 mL of the dialyzed solution, add 20 µg of trypsin, and incubate for 6–8 h at room temperature. Again add 20 µg of trypsin, and incubate for 16–18 h for a total of 24 h of incubation of the trypsin-treated solution.

[NOTE—Store the *Standard solution* in a freezer.]

Sample solution: Using an accurately weighed quantity of Alteplase, proceed as directed in the *Standard solution*.

Chromatographic system

(See [Chromatography \(621\)](#), *System Suitability*.)

Mode: LC

Detector: UV 214 nm

Column: 4.6-mm × 10-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 100 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 1.5 between peaks 6 and 7 as defined by the [USP Alteplase RS](#) Data Sheet. The times for peaks 6 and 7 baseline widths are NMT 0.5 min.

Analysis

Samples: *Standard solution*, *Sample solution*, and a mixture of the *Standard solution* and the *Sample solution* (1:1)

Measure the responses for NLT 20 major peaks as defined in the [USP Alteplase RS](#) Data Sheet.

Acceptance criteria: The retention times of the corresponding peaks of the *Standard solution* and the *Sample solution* do not differ by more than 0.4 min, and the peak area ratios relative to peak 19 (as shown on the [USP Alteplase RS](#) Data Sheet) do not differ by more than 20%.

No additional significant peaks or shoulders are found, a significant peak or shoulder being defined as one having a peak response of NLT 5% of peak 19.

ASSAY

• BIOLOGICAL POTENCY

Buffer: 1.38 mg/mL of monobasic sodium phosphate, 7.10 mg/mL of anhydrous dibasic sodium phosphate, 0.20 mg/mL of sodium azide, and 0.10 mg/mL of polysorbate 80 in water

Human thrombin solution: 33 U.S. Units in terms of the U.S. Standard Thrombin/mL in *Buffer*

Human fibrinogen solution: 2 mg/mL of human fibrinogen in *Buffer*

Human plasminogen solution: 1 mg/mL of human plasminogen in *Buffer*

Standard stock solution: 1.0 mg/mL (580,000 USP Alteplase Units) of [USP Alteplase RS](#) in water

Standard solutions: Dilute volumes of *Standard stock solution* with water to obtain a series of five *Standard solutions* having known concentrations ranging from 145 to 9.3 USP Alteplase Units/mL.

Sample stock solution: 1.0 mg/mL of Alteplase in water

Sample solutions: Dilute a volume of *Sample stock solution* with *Buffer* to obtain a series of dilutions of about 1:20,000; 1:10,000; and 1:5,000.

Analysis

Samples: *Standard solutions* and *Sample solutions*

To a set of labeled glass test tubes add 0.5 mL of *Human thrombin solution*. To separate test tubes add 0.5 mL of each *Standard solution* or *Sample solution*, and store on ice. To a second set of labeled glass tubes, add 20 µL of *Human plasminogen solution* and 1 mL of *Human fibrinogen solution*, and store on ice. Beginning with the thrombin–*Standard solution* mixture containing the *Standard solution* with the lowest number of USP Units/mL, record the time, and separately add 200 µL of each of the thrombin–*Standard solution* mixtures to the test tubes containing the plasminogen–fibrinogen mixture. Using a vortex mixer, intermittently mix the contents of each tube for a total of 15 s, and carefully place into a rack in a 37° circulating water bath. A visually turbid clot forms within 30 s, followed by the formation of bubbles within the clot. Record the clot lysis time (t_c) from the first addition of the Alteplase solution to the last bubble to rise to the surface.

Using a least squares fit, determine the equation of the line using the log values of the standard concentration, in USP Alteplase Units/mL, versus the log values of their clot lysis times in s taken:

$$\log t = m(\log U_s) + b$$

t = time to bubble release (s)

m = slope of the line