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rAlbumin Human

DAHKSEVAHR FKDLGEENFK ALVLIAFAQY LQQCPFEDHV KLVNEVTEFA
KTCVADESAE NCDKSLHTLF GDKLCTVATL RETYGEMADÇ ÇAKQEPERNE
ÇFLQHKDDNP NLPRLVRPEV DVMCTAFHDN EETFLKKYLY EIARRHPYFY
APELLFFAKR YKAAFTEÇÇQ AADKAACLLP KLDELREDEGK ASSAKQRLKC
ASLQKFGERA FKAWAVARLS QRFPAEFAE VSKLVTDLTK VHTEÇÇGDL
LEÇADDRADL AKYIÇENQDS ISSKLKEÇÇE KPILLEKSHCI AEVENDEMPA
DLPSLAADFV ESKDVCKNYA EAKDVFLGMF LYEYARRHPD YSVVLLRLA
KTYETTLEKÇ ÇAAADPHEÇY AKVFDEFKPL VEEPQNLIKQ NÇELFEQLGE
YKFQNALLVR YTKKVPOVST PTLVEVSRNL GKVGSKÇÇKH PEAKRMPÇAE
DYLSVVLNQL ÇVLHEKTPVS DRVTKÇÇTES LVNRRPÇFSA LEVDETYVPK
EFNAETFTFH ADIÇTLSEKE RQIKKQTALV ELVKHKPKAT KEQLKAVMDD
FAAFVEKÇÇK ADDKETÇFAE EGKKLVAASQ AALGL

C₂₉₃₆H₄₆₂₄N₇₈₆O₈₈₉S₄₁ 66,438 Da

DEFINITION

Recombinant Albumin Human (rAlbumin Human or rHA) is produced by recombinant DNA expression in *Saccharomyces cerevisiae*. Structural equivalence (primary, secondary, and tertiary) between rHA and human serum albumin (HSA) has been demonstrated. It consists of three domains composed of 585 amino acids containing a single tryptophan (Trp₂₁₄), one free thiol (Cys₃₄), and 17 disulfide bridges. It is presented as a sterile and nonpyrogenic aqueous liquid consisting of a 10% (w/v) or 20% (w/v) solution in Water for Injection. No human- or animal-derived raw material is involved in its manufacture. It contains NLT 95% and NMT 105% of the labeled amount and NLT 99% of its total protein is albumin. It contains no added antimicrobial agents, but it may contain appropriate stabilizing agents. The presence of process-related impurities, host cell DNA, and host cell proteins is process specific; suitable limits should be determined by appropriately validated methods. However, the limit for host cell proteins should be NMT 0.15 µg/g.

IDENTIFICATION

• A. PEPTIDE MAPPING

[NOTE—See [Biotechnology-Derived Articles—Peptide Mapping \(1055\)](#) for guidance.]

Tris buffer: 0.1 M tris(hydroxymethyl)aminomethane. Adjust with hydrochloric acid to a pH of 8.0.

Dilute Tris buffer: *Tris buffer* and water (50:50)

Solution A: Trifluoroacetic acid and water (1:1000)

Solution B: To 350 mL of acetonitrile add 150 mL of water and 425 µL of trifluoroacetic acid.

Dithiothreitol solution: 0.1 M dithiothreitol

Iodoacetamide solution: 0.1 M iodoacetamide in *Tris buffer*

Trypsin solution: 1 mg/mL of trypsin in 10 mM hydrochloric acid

EDTA solution: 0.1 M ethylenediaminetetraacetic acid (EDTA) in water

Diluent: To 5.76 g of guanidine hydrochloride add 5 mL of *Dilute Tris buffer* and 200 µL of *EDTA solution*. Dilute with *Dilute Tris buffer* to a final volume of 10 mL.

Standard solution: Add 20 µL of [USP rAlbumin Human RS](#) to 80 µL of *Diluent*. Add 5 µL of *Dithiothreitol solution*, and incubate at 37° for 75 min. Add 10 µL of *Iodoacetamide solution*, and incubate for an additional 75 min at 37° in the dark. Add 100 µL of *Dilute Tris buffer*, 400 µL of water, and 10 µL of *Trypsin solution*, and incubate at 37° with shaking for 24 h. Centrifuge, and dilute a portion of the supernatant in *Solution A* (50:50).

Sample solution: 50 mg/mL of rAlbumin Human in water. To 20 µL of this solution add 80 µL of *Diluent*. Add 5 µL of *Dithiothreitol solution*, and incubate at 37° for 75 min. Add 10 µL of *Iodoacetamide solution*, and incubate for an additional 75 min at 37° in the dark. Add 100 µL of *Dilute Tris buffer*, 400 µL of water, and 10 µL of *Trypsin solution*, and incubate at 37° with shaking for 24 h. Pulse centrifuge, and dilute a portion of the supernatant in *Solution A* (50:50).

Mobile phase: See [Table 1](#).

Table 1

Time (min)	Solution A (%)	Solution B (%)	Flow Rate (mL/min)
0	95	5	0.5
5	95	5	0.5
75	60	40	0.5
100	40	60	0.5
104	0	100	0.5
108	0	100	0.5
109	95	5	1.0
115	95	5	1.0
116	95	5	0.5
120	95	5	0.5

Chromatographic system(See [Chromatography \(621\), System Suitability.](#))**Mode:** LC**Detector:** UV 214 nm**Column:** 4.6-mm × 25-cm; 5-µm packing L1**Column temperature:** 35°**Flow rate:** See [Table 1.](#)**Injection volume:** 100 µL**Analysis****Samples:** *Standard solution* and *Sample solution***Acceptance criteria:** The peptide map chromatographic profiles of the *Sample solution* are similar to those of the *Standard solution*.**• B. ELECTROSPRAY MASS SPECTROMETRY****Solution A:** Trifluoroacetic acid and water (1:1000)**Solution B:** To 140 mL of acetonitrile add 60 mL of water and 180 µL of trifluoroacetic acid.**Solution C:** Acetonitrile and water (50:50)**Solution D:** To 5 mL of *Solution C* add 10 µL of formic acid.**System suitability solution:** Dissolve 2 mg of horse heart myoglobin in 589 µL of Water for Injection. Dilute 25 µL of this solution with 475 µL of *Solution D*.**Sample solution:** 10 mg/mL of rAlbumin Human in water**Mobile phase:** See [Table 2.](#)**Table 2**

Time (min)	Solution A (%)	Solution B (%)
0	95	5
5	95	5
10	0	100
15	0	100

Chromatographic system(See [Chromatography \(621\), System Suitability.](#))**Mode:** LC**Detector:** UV 280 nm**Column:** 2.1-mm × 3-cm; desalting cartridge, equilibrated with *Solution C*¹**Flow rate:** 0.2 mL/min