Calculate the purity of the *Sample solution*:

$$\text{Result} = 100 - C_I$$

C_I = mean of the percentages of contaminant levels found in *Lanes 8* and *9* (all the bands other than the albumin band), disregarding any band due to the *Diluted sample buffer*

Acceptance criteria: *Sample solution* purity is NLT 99.0%. [NOTE—The main albumin band is not quantitated. See the test for *Total Protein*.]

- **TOTAL PROTEIN**

Sodium chloride solution: 0.15 M sodium chloride in water

Copper sulfate solution: 60 mg/mL of copper sulfate pentahydrate and 600 mg/mL of potassium sulfate[6] in sulfuric acid low in nitrogen

Sample solution: Dilute 0.5 g of rAlbumin Human with 2.5 mL of *Sodium chloride solution* (equivalent to about 3.3 mg/mL of total protein).

Blank: 33.3 mg/mL of glycine in *Sodium chloride solution*

Analysis: To 3.0 mL of the *Sample solution* and the *Blank*, in suitable distillation tubes, add 5 mL of *Copper sulfate solution*. Incubate at 420° for a minimum of 2 h, or until the residues appear white. When the solutions are cool, transfer the residues quantitatively with a minimum quantity of water to a micro-Kjeldahl flask, and determine the residues, using *Nitrogen Determination* ⟨461⟩, *Method II*. Multiply the result, corrected for the *Blank* and for the specific gravity of the *Sample solution*, by 6.25 to calculate the quantity of protein.

Acceptance criteria: 95%–105% of the quantity of protein stated on the label

OTHER COMPONENTS

- **SODIUM CONTENT**

Diluent: 1.0 mg/mL of cesium chloride in water

Standard solutions: Prepare 0.5, 1.00, 1.50, and 2.00 mg/mL of sodium chloride in *Diluent*.

Sample solution: 80 µg/mL of rAlbumin Human in *Diluent*

Apparatus

Mode: Atomic absorption

Emission wavelength: 589 nm

Analysis: [NOTE—Use peak area measurements for quantitation.]

Samples: *Diluent* (as blank), *Standard solutions*, and *Sample solution*

Introduce a blank solution (*Diluent*) into the atomic generator, and adjust the instrument reading to zero. Determinations are made by comparison with the *Standard solutions* of known concentration. If the *Sample solution* emission exceeds that of the *Standard solutions* with the highest concentration, dilute the *Sample solution* with *Diluent*. Introduce the most concentrated *Standard solution* into the instrument, and adjust the sensitivity to obtain a suitable reading. Introduce the *Sample solution* and *Standard solutions* into the instrument at least three times, and record the steady reading. Rinse the apparatus with blank solution each time, and ascertain that the reading returns to its initial blank value. Plot the mean of the readings obtained for the *Standard solutions* against their respective sodium concentrations. From the standard curve, calculate the sodium concentration content in the *Sample solution*, and adjust for the specific gravity of the rAlbumin Human (see *Total Protein*).

[6] Copper sulfate pentahydrate and potassium sulfate tablets (each tablet with 1.5 g of K_2SO_4 + 0.15 g of $CuSO_4 \cdot 5H_2O$) are available from Foss (No. 15270054).

Acceptance criteria: 120–160 mM sodium

IMPURITIES

- **LIMIT OF HIGH MOLECULAR WEIGHT PROTEINS**

Solution A: 200 mg/mL of sodium azide

Buffer: Dissolve 54.2 g of dibasic sodium phosphate dihydrate, 30.0 g of monobasic sodium phosphate dihydrate, and 284.0 g of anhydrous sodium sulfate in 1600 mL of water. Add 50 mL of *Solution A*, and dilute with water to 2000 mL.

Mobile phase: *Buffer* and water (10:90)

Sample solution: 40 mg/mL of rAlbumin Human

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 7.8-mm × 30-cm; 5-µm packing L59

Flow rate: 1.0 mL/min

Injection volume: 50 µL. [NOTE—The peak due to high molecular weight impurities, such as the polymer of albumin, appears in the void volume of the chromatogram.]

Analysis

Sample: *Sample solution*

Calculate the percentage of albumin polymer in the sample:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of albumin polymer

r_T = sum of all rAlbumin Human related peak responses

Acceptance criteria

Individual impurities: NMT 1.0%

SPECIFIC TESTS

- **pH** ⟨791⟩

Sample solution: 1% (w/v) protein solution diluted with 0.9% (w/v) sodium chloride

Acceptance criteria: 6.4–7.4

- **STERILITY TESTS** ⟨71⟩**:** Meets the requirements
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** NMT 0.5 USP Endotoxin Unit/mL of rAlbumin Human

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight glass containers, and store at 2°–8°. Do not freeze.
- **LABELING:** Label to indicate that the material is of recombinant DNA origin.

Change to read:

- **USP REFERENCE STANDARDS** ⟨11⟩

USP rAlbumin Human RS

• (CN 1-May-2018)

Alcohol—*see Alcohol General Monographs*

Diluted Alcohol

DEFINITION

Diluted Alcohol is a mixture of Alcohol and water containing NLT 41.0% and NMT 42.0% by weight, corresponding to NLT 48.4% and NMT 49.5% by volume, at 15.56°, of C_2H_5OH.

Diluted Alcohol may be prepared as follows.

Alcohol	500 mL
Purified Water	500 mL