**HUMAN ALBUMIN SOLUTION**

**Definition:**

Human albumin solution is an aqueous solution of albumin component obtained from human plasma that complies with the requirement of the monograph on plasma for fractionation.

**Production:**

Separation of albumin is carried out under control conditions particularly on pH, Ionic strength and temperature so that in the final product not less than 95% of total protein is albumin. Human albumin is prepared as a concentrated solution containing 150 - 250 g/l of total protein or as an isotonic solution containing 35 - 50 g/l of total protein. No antimicrobial agent is added at any stage during preparation and all processing steps are conducted in a manner to minimize the risk of contamination from either microorganism or other deleterious matter. The solution is sterilized by filtration and distribute aseptically in sterile containers which are closed so as to prevent contamination. Then solution is heated and then maintained at 60º C ± 0.5º C for 10 hours. Finally the containers are incubated at 30 -32ºC for not less than 14 days or at 20 -25ºC for not less than 4 weeks and examined visually. Those showing abnormalities such abnormal colour, turbidity, microbial contamination or presence of a typical particle must be discarded.

**Description (Characters):**

A clear, slightly viscous liquid, it is almost colourless to greenish yellow or amber, depending on protein concentration and the method of fractionation used.

**Identification:**

A. Immunoelectrophoresis technique:

Examine by a suitable immunoelectrophoresis technique. Using antisera to normal human serum, compare normal human serum and preparation under examination, both diluted to contain 1.0 per cent w/v of protein. The main component of the preparation under examination corresponds to the Albumin component of normal human serum.

B. Double Immuno Diffusion:

Precipitation test with a suitable range of species specific antisera which gives positive results for the presence of protein of human origin and negative results with antisera specific to plasma protein for other species.

**pH (2.4.24):**

Dilute the preparation to be examined with a 9 g/L solution of sodium chloride R to produce a solution containing 10 g/L of protein; pH of the resulting solution 6.7 – 7.3.

**Haem content:**

Dilute the preparation to be examined using a 9 g/L solution of sodium chloride to obtain a solution containing 10 g/L of protein. The absorbance of resulting solution shall be not more than 0.15 (2.4.7) when measured by spectrophotometer at 403 nm.

**Molecular Size Distribution:**

**Liquid Chromatography (2.4.14):**

*Test solution;* dilute the preparation to be examined with a 9 g/L solution of sodium chloride to concentration suitable for the chromatographic system used. Concentration in the range of 4 - 12 g/L and injection of 50 – 600 µg of protein are usually suitable.

**Column:**

Size: length = 0.6 m, φ = 7.5 mm or length = 0.3 m, φ = 7.8 mm

Stationary phase: Hydrophilic silica gel for chromatography, of a great suitable for fractionation of globular proteins with relative molecular masses
Mobile phase: Dissolve 4.873 g of disodium hydrogen phosphate dehydrate, 1.741 g of sodium dihydrogen phosphate monohydrate, 11.688 g of sodium chloride and 50 mg of sodium azide in 1 L of water.

Flow rate: 0.5 ml / minute

Detection: spectrophotometer at 280 nm

The peak due to polymers and aggregates is located in the part of the chromatogram representing the void volume. Disregard the peak due to the stabilizer, the area of the peak due to polymers and aggregate is not greater 10% of the total area of the chromatogram.

Protein Composition:

Zone electrophoresis (2.4.12):

Use strips of suitable cellulose acetate gel or agarose gel as the supporting medium and barbital buffer solution pH 8.6 as the electrolyte solution.

If cellulose acetate is the supporting material, the method described below can be used. If agarose gels are used, and because they are normally part of an automated system, the manufacturer’s instructions are followed instead.

Test solution: Dilute the preparation to be examined with a 9 g/L solution of sodium chloride R to a protein concentration of 20 g/L.

Reference solution: Dilute human albumin for electrophoresis BRP with a 9 g/L solution of sodium chloride R to a protein concentration of 20 g/L.

To a strip apply 2.5 µL of the test solution as a 10 mm band or apply 2.5 µL per millimeter if a narrower strip is used.

To another strip, apply in the same manner the same volume of the reference solution. Apply suitable electric field such that the most rapid band migrates at least 30 mm. Treat the strips with amido black 10 B solution R for 5 minutes. Decolourise with the mixture of 10 volumes of glacial acetic acid R and 90 volumes of methanol R until the background is just free of colour. Develop the transparency of the strips with the mixture of 19 volumes of glacial acetic acid R and 81 volume of methanol R. measure the absorbance of the band at 600 nm in an instrument having a linear response over the range of measurement. Calculate the result as the mean of 3 measurements of each strips.

System suitability In the electropherogram obtained with the reference solution on cellulose acetate or on agarose gel, the proportion of protein in the principal band is within the limits stated in the leaflet accompanying the reference preparation.

Results: In the electropherogram obtained with the test solution on cellulose acetate or on agarose gel, not more than 5% of the protein has a mobility different from that of the principal band.

Prekallikrein activator

Maximum 35 IU/mL using the method as described in the Test for prekallikrein activator in the monograph for Human normal immunoglobulin for intravenous use

Pyrogens (2.2.8) / or Bacterial Endotoxin (BET) (2.2.3)

It complies with the test for pyrogen or, preferably and where justified and authorized with a validated in-vitro test such as the Bacterial Endotoxin test.
**Pyrogens:**
Complies with the test for pyrogen using 3 mL/kg of the rabbit weight irrespective of the protein content, in rabbit that have not received blood products” or

**Bacterial Endotoxin (BET)**
Where the bacterial endotoxin test is used, the preparation to be examined contains less than 0.5 IU of endotoxin per milliliter for solution with a protein content not greater than 50 g/L, less than 1.3 IU of endotoxin per milliliter for solution with a protein content greater than 50 g/L but not greater than 200 g/L, and less than 1.7 IU of endotoxin per milliliter for solution with a protein content greater than 200 g/L but not greater than 250 g/L.”

**Sterility (2.2.11):**
Complies with the tests for sterility.

**Abnormal Toxicity (2.2.1):**
Complies with the test for abnormal toxicity, using method B and 0.5 ml of the solution for each mouse and 5 ml for each guinea pig irrespective of the protein content.

**Assay For protein**
Dilute to about 0.75% w/v of total protein with saline solution. Take 2 mL of the solution in a round bottom centrifuge tube. Add 2 mL of 7.5% w/v solution of sodium molybdate and 2 mL of mixture of 30 volumes of water and 1 volume of nitrogen free sulphuric acid.

Shake, centrifuge for 5 minutes. Decant the supernatant liquid and let the inverted tube stand on a filter paper to drain the fluid. Carry out method F for determination of nitrogen (2.3.30) on the residue thus obtained and multiply the result by 6.25 to obtain the protein content.

For Sodium:
Dilute to 0.01 % w/v of protein with water and determine by Method A (2.4.2) for atomic absorption spectrophotometer, or by Method B for flame photometry (2.4.4), measuring at about 589 nm and using sodium solution FP suitably diluted with water as the standard solution.

For potassium:
Dilute To 0.25 % w/v of protein with water and determine by Method A (2.4.2) for atomic absorption spectrophotometry, or by Method B for flame photometry (2.4.4), measuring at about 767 nm and using potassium solution FP suitably diluted with water as the standard solution.

**Human albumin intended for administration to patients undergoing dialysis or to premature infants complies with the following additional test.**

**Aluminium(2.3.8).**
Not more than 200 µg of Al per liter. Determine by atomic absorption spectrophotometry (2.4.2), with a furnace as a atomic generator and measuring at 309.3nm and using as standard solutions a suitable range of dilutions in water of aluminium standard solution (10ppm Al) further diluted, as necessary with a solution containing 0.17% w/v of Magnesium nitrate and 0.05% w/v of Octoxinol 10 in a solution of nitric acid containing 1% w/v of nitric Acid. Prepare suitable dilutions of the preparations under examination and human albumin for Aluminium validation RS with water. Dilute the solutions, as necessary, with the magnesium nitrate – Octoxinol 10-nitric acid solution used for dilution of the standard solution. The test is valid only if the Aluminium content determined for Human albumin for Aluminium validation RS is within 20% of the stated value.
NOTE: Wash all equipment with a solution containing 20.0% w/v of nitric acid before use and use plastic containers only to prepare solutions

Storage:

Protected from light at a temperature between 2° and 25°C. Human albumin stored at 2-8°C may be expected to continue to meet the requirements of the monograph for 5 years from the date on which it was heated at 60°C for 10 hours. Human albumin stored at a temperature not exceeding 25°C may be expected to continue to meet the requirements of the monograph for 3 years from the date on which it was heated at 60°C for 10 hours.

Labeling:

The label states:
- the name of the preparation;
- the volume of the preparation;
- the content of protein expressed in grams per litre;
- the content of sodium expressed in millimoles per litre;
- that the product is not to be used if it is cloudy or if a deposit has formed;
- the name and concentration of any added substances (for example stabilizer);
- that the contents must not be used more than 4 hours after the container has been penetrated and any remnant portion must be discarded
- Storage condition
- the date after which the solution is not intended to be used
- either that the preparation is suitable for administration to patients undergoing dialysis or to premature infants or that it is not intended for such purpose