HUMAN PLASMA PROTEIN FRACTION

Human plasma protein fraction is a sterile isotonic aqueous solution of proteins of plasma or serum containing albumin and globulins. It is prepared as an isotonic solution containing 4.0 to 5.0% w/v of total protein. It contains no fibrinogen or antibodies.

PRODUCTION

The separation of protein may be done by precipitation with suitable organic solvents under controlled conditions, particularly of pH, ionic strength and temperature, so that in the final product not less than 85% of the total protein as albumin. Residual solvents, if present, may be removed by freeze drying or other suitable treatment. Alternative methods of preparation which shall not affect the integrity of the product and shall have been shown to yield consistently a product which is safe for intravenous injection may be adopted.

The product is dissolved in water and sufficient quantities of suitable stabilizer against the effect of heat, like sodium caprylate in a suitable concentration, and sufficient sodium chloride to adjust the sodium ions to between 130 and 160 millimoles per litre may be added but no antibiotic or antimicrobial preservative is added at any during preparation. The solution is sterilized by filtration through a bacteria retentive filter and distributed aseptically into sterile containers which are then closed so as to prevent microbial contamination. The solution in its final container is heated at a 60±0.5° and maintained at this temperature for 10 hours. The containers are then incubated at 30°-32° for not less than 14 days or at 20°-25°C for not less than 4 weeks and examined visually for evidence of microbial contamination. Those showing abnormalities such as abnormal color, turbidity, presence of atypical particles or microbial contamination are discarded.

Human plasma protein fraction contains not less than 95% and not more than 105% of the stated amount of total protein.

DESCRIPTION

A clear, almost colorless or pale yellow liquid; almost odourless. On storage a dust-like precipitate may develop but it disappears on shaking.

Identification:

A. Immunoelectrophoresis technique:

Examine by a suitable immunoelectrophoresis technique. Using antiserum 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 (2.2.24):

Dilute with sufficient saline solution to produce a solution containing 1% w/v of protein; pH of resulting solution 6.7 – 7.3.

Molecular Size Distribution:

Liquid Chromatography (2.4.16)

**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 = 0.6m, Ø = 7.5 mm or length = 0.3m, Ø = 7.8mm

**Stationary phase:** Hydrophilic silica gel for chromatography, of a great suitable for fractionation of globular proteins with relative molecular masses in the range 10,000 – 500,000 Da.

**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 15% of the protein is contained in the bands other than the principal band.

**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 nor more than 0.15 (2.4.7) when measured by spectrophotometer at 403 nm.

**Sterility (2.2.11):**

Complies with the tests for sterility.

**Abnormal toxicity (2.2.1):**

Complies with the tests 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.

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

It complies with the test for pyrogens 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 pyrogens 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.”

**Assay:**

*For Total 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 residues thus obtained and multiply the result by 6.25 to obtain the protein content.

**Sodium**

Not less than 95% and not more than 105% of the stated amount and, in any case not more than 160 mM millimoles of sodium per litre, determine by Method A for atomic absorption spectrophotometry (2.4.2), measuring at 589nm and using sodium solution AAS suitably diluted with water to prepare the standard solutions.

**Potassium**

Not more than 50µmol of K per g of protein determine by atomic absorption spectrophotometry (2.4.2), measuring at 766nm and using sodium solution AAS suitably diluted with water to prepare the standard solutions.

**Storage**

Store protected from light at a temperature between 2º and 25º.

**Labelling:**

The label states (1) the volume in the container; (2) the total amount of protein in the container expressed in g per litre or as percentage; (3) the concentration of sodium and potassium ions expressed in millimoles per litre; (4) the names and concentrations of any stabilizing agents and any other additives in the final solution; (5) the type of source material used to manufacture the product; (6) the words “do not use if turbid”; (7) that the contents must not be used more than 4 hours after the container has been penetrated and any remnant portion must be discarded; (8) the storage conditions.