Albumin Reagent
BCG Method

PRODUCT SUMMARY

Stability	:	Until Expiry at 2-25°C
Linear Range	:	Up to 60 g/L (6.0 g/dL)
Specimen Type	:	Serum
Method	:	Endpoint
Reagent Preparation	:	Supplied ready to use.

IVD

SYMBOLS IN PRODUCT LABELLING

EC REP	Authorized Representative		Temperature Limitation
IVD	For in vitro diagnostic use		Use by/Expiration Date
LOT	Batch code/Lot number		CAUTION. CONSULT INSTRUCTIONS FOR USE.
REF	Catalogue number		Manufactured by
	Consult instructions for use		Exclamation Mark

INTENDED USE
This reagent is intended for the in vitro quantitative determination of Albumin in human serum.

CLINICAL SIGNIFICANCE[1]
Albumin is quantitatively the major single contributor to the plasma total protein and performs a number of functions including:
(i) Regulation of the distribution of extracellular fluid
(ii) Acts as a transport agent for a wide variety of substances such as hormones, lipids, vitamins, calcium and trace metals and
(iii) Forms part of the amino acid pool.
Measurement of total protein levels alone may be misleading, and may be normal in the face of quite marked changes in the constituent proteins. For example - a fall in albumin may roughly be balanced by a rise in immunoglobulin levels. This is quite a common combination.
True hyperalbuminaemia probably does not occur and an increase in albumin concentration is usually only encountered in dehydration due to the reduced plasma water content or artefactually, as a result of venous stasis during venipuncture (Most common cause).
Hypoalbuminaemia occurs as a result of -
(i) Overhydration due to water excess,
(ii) Excessive protein loss through the skin after severe burns, from the kidney in the nephrotic syndrome and through the intestine in protein losing enteropathy,
(iii) Decreased synthesis due to dietary deficiency, liver disease or malabsorption or,
(iv) Increased catabolism.

METHODOLOGY
Several procedures are currently available for the determination of albumin and include dye binding methods, electrophoresis, immunological and salt fractionation.
The most commonly used procedures are the dye binding methods of which Bromocresol Green (BCG) is the most popular. However, one of the major drawbacks of this method is its lack of specificity. Despite the many published modifications, existing BCG methods still tend to overestimate low concentrations of albumin[2,3] due to non specific reactions with other plasma proteins.
This kit is based on the method of Doumas et al[4] in which albumin binds with BCG causing a shift in the absorption spectra of the dye. The dye -albumin complex formed has an absorbance peak at 625nm which is proportional to the concentration of albumin in the sample.

REAGENT COMPOSITION

Active Ingredients	Concentration
Succinate buffer	90 mmol/L
Bromocresol Green	0.26 mmol/L

pH 4.10 ± 0.1 at 20°C

Reagent also contains surfactant and stabilizers necessary for optimum reagent performance.

Hazard Symbol: Exclamation Mark
Signal Word: Warning
Hazard Statements
H319 Causes serious eye irritation
Precautionary Statements - Prevention
Wash face, hands and any exposed skin thoroughly after handling
Wear protective gloves/protective clothing/eye protection/face protection
Wear eye/face protection
Precautionary Statements - Response
Eyes
IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing
If eye irritation persists: Get medical advice/attention
Precautionary Statements - Storage
None
Precautionary Statements - Disposal
None
Hazards not otherwise classified (HNOC)
Not applicable
Unknown Toxicity
0.0185% of the mixture consists of ingredient(s) of unknown toxicity
Other information
Harmful to aquatic life
Interactions with Other Chemicals
No information available.
Refer to the product Safety Data Sheet for additional information.

REAGENT PREPARATION
The reagent is supplied ready to use.

STABILITY AND STORAGE
When stored at 2 - 25°C the reagent is stable until the expiration date stated on the bottle and kit box label.

Indications of Reagent Deterioration:
- Turbidity;
- Presence of a precipitate; and/or
- Failure to recover control values within the assigned range.

SPECIMEN COLLECTION AND HANDLING
Serum: Use non-haemolysed serum collected without prolonged venous stasis.
Storage: Specimens are stable for at least 20 days when stored at 4°C.

ADDITIONAL EQUIPMENT REQUIRED BUT NOT PROVIDED
- If required, pipettes for accurately dispensing measured volumes.
- A clinical chemistry analyzer capable of maintaining constant temperature (37°C) and measuring absorbance at 630nm.
- Analyzer specific consumables, eg: sample cups.
- Normal and Abnormal assayed control material.
- Calibrator or suitable aqueous Albumin standard.

ASSAY PROCEDURE
These instructions are for manual instrumentation but can be adapted to most automated instruments. Specific instructions are available upon request.

SYSTEM PARAMETERS

Temperature	37°C
Wavelength	630nm
Assay Type	Endpoint
Direction	Increase
Sample : Reagent Ratio	1:100
eg: Sample Vol	3µL
Reagent Vol	300µL
Incubation Time	90 seconds
Reagent Blank Limits	Low 0.0 AU
(630nm, 1cm lightpath)	High 2.0 AU
Linearity	0 - 60 g/L (0.0 - 6.0 g/dL)
(refer to linearity section)	
Analytical Sensitivity	0.03ΔAbs per g/L
(630nm, 1cm lightpath)	(0.3ΔAbs per g/dL)

CALCULATIONS
Results are calculated, usually automatically by the instrument, as follows:

Thermo SCIENTIFIC

$$\text{Albumin} = \frac{\text{Absorbance of Unknown}}{\text{Absorbance of Calibrator}} \times \text{Calibrator Value}$$

Example:

Absorbance of calibrator = 1.2
Absorbance of unknown = 0.62
Value of calibrator = 40 g/L (4.0 g/dL)

$$\text{Albumin (g/L)} = \frac{0.62}{1.2} \times 40 = 21 \text{ g/L}$$

$$\text{Albumin (g/dL)} = \frac{0.62}{1.2} \times 4.0 = 2.1 \text{ g/dL}$$

NOTES

1. The reagent and sample volumes may be altered proportionally to accommodate different spectrophotometer requirements.
2. The temperature of the reaction is not critical, however the temperature of the spectrophotometer should be held constant.
3. Final absorbance readings should be taken less than 90 seconds after sample addition.
4. Decreasing the sample volume to reagent volume ratio to 1:200 will increase the observed linearity to 100 g/L or 10 g/dL. Subsequently, sensitivity will be reduced.
5. Unit conversion: g/L x 0.1 = g/dL.

CALIBRATION

Calibration is required. A suitable bovine or human Albumin Standard(s) or a serum based calibrator, with an assigned value traceable to a primary standard (eg NIST or IRMM) is recommended. For calibration frequency on automated instruments, refer to the instrument manufacturers specifications.
However, calibration stability is contingent upon optimum instrument performance and the use of reagents which have been stored as recommended in the stability and storage section of this package insert. Recalibration is recommended at anytime if one of the following events occurs:-

- The Lot number of reagent changes.
- After preventative maintenance is performed or a critical component is replaced.
- Control values have shifted or are out of range and a new vial of control does not rectify the problem.

QUALITY CONTROL

To ensure adequate quality control, normal and abnormal control with assayed values should be run as unknown samples:-

- At least every eight hours.
- When a new bottle of reagent is used.
- After preventative maintenance is performed or a critical component is replaced.

Control results falling above the upper limit or below the lower limit of the established ranges indicate the assay may be out of control.
The following corrective actions are recommended in such situations:-

- Repeat the same controls.
- If repeated control results are outside the limits, prepare fresh control serum and repeat the test.
- If results are still out of control, recalibrate with fresh calibrator, then repeat the test.
- If results are still out of control, perform a calibration with fresh reagent, then repeat the test.
- If results are still out of control, contact Technical Services or your local distributor.

LIMITATIONS

1. Studies to determine the level of interference from haemoglobin, bilirubin and lipaemia were carried out and the following results were obtained:
   Haemoglobin: No interference from haemoglobin up to 540mg/dL.
   Bilirubin: No interference from bilirubin up to 340µmol/L (20mg/dL).
   Lipaemia: No interference from lipaemia, measured as triglycerides, up to 15.7 mmol/L (1380mg/dL).
2. Young DS[5] has published a comprehensive list of drugs and substances which may interfere with this assay.

EXPECTED VALUES[6]

Ambulant Male	35-48g/L (3.5 - 4.8 g/dL)
Ambulant Female	33-45g/L (3.3 - 4.5 g/dL)

In non ambulatory hospitalised patients the haemodilution of recumbency may reduce the albumin levels by up to 5g/L. The quoted values were derived from non selected male (100) and female (100) blood donors and should serve as a guide only. It is recommended that each laboratory verify this range or derives a reference interval for the population that it serves.[7]

PERFORMANCE DATA

The following data was obtained using the Albumin reagent on an automated clinical chemistry analyzer.

IMPRECISION:

Imprecision was evaluated using two levels of commercial control and following the NCCLS EP5-T procedure.[8]

Within Run	LEVEL I	LEVEL II
Number of data points	80	80
Mean (g/L / g/dL)	28 / 2.8	44 / 4.4
SD (g/L / g/dL)	0.47 / 0.05	0.6 / 0.06
CV (%)	1.7	1.5
Between Day	LEVEL I	LEVEL II
Number of data points	80	80
Mean (g/L / g/dL)	28 / 2.8	40 / 4.0
SD (g/L / g/dL)	0.6 / 0.06	1.1 / 0.11
CV (%)	2.1	2.5

METHOD COMPARISON

Comparison studies were carried out using another commercially available BCG method for Albumin as a reference. Normal and abnormal human serum samples were assayed in parallel and the results compared by least squares regression. The following statistics were obtained.

Number of sample pairs	55
Range of results	7 - 48 g/L (0.7 - 4.8 g/dL)
Slope	0.935
Intercept	1.7 g/L (0.17 g/dL)
Correlation coefficient	0.979

LINEARITY

When run as recommended the assay is linear to 60 g/L (6.0 g/dL).

ANALYTICAL SENSITIVITY

When run as recommended the sensitivity of the assay is 0.03ΔA per g/L (0.3ΔA per g/dL).

REFERENCES

1. "Plasma Proteins and Immunoglobulins" in Clinical Chemistry in Diagnosis and Treatment. Lloyd-Luke 1979; Chap XIV:305-10.
2. Ferreria P, Price CP. Clin Chem Acta 1974;55:259.
3. Webster D, Clin Chem 1974;53:109.
4. Doumas BT, Arends RL, Pinto PC in Standard Methods of Clinical Chemistry 1972; 7:175-189
5. Young DS, Effects of Drugs on Clinical Laboratory Tests. Third Edition 1990;12-6.
6. Tietz Textbook of Clinical Chemistry and Molecular Diagnosis (4th Ed.) Burtis, Ashwood & Bruns (Eds), Elsevier Saunders, 2005; 2254.
7. Wachtel M et al, Creation and Verification of Reference Intervals. Laboratory Medicine 1995; 26:593-7.
8. National Committee of Clinical Laboratory Standards. User evaluation of Precision Performance of Clinical Chemistry Devices NCCLS 1984; NCCLS publication EP5-T.

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REF

Reorder Information

Catalogue No.	Configuration
TR36026/1105-500	2 x 250 mL