

THE WORLD'S FOREMOST MANUFACTURER OF RESEARCH BIOCHEMICALS AND DIAGNOSTIC REAGENTS TELEPHONE: USA/CANADA 1-800-325-3010 OUTSIDE USA/CANADA call COLLECT 314-771-5750

> FAX: USA/CANADA 1-800-325-5052 OUTSIDE USA/CANADA 314-771-5757 INTERNET: sigma@sial.com

## **ALBUMIN, BOVINE**

**CAS NUMBER:** 9048-46-8

SYNONYMS: Bovine Serum Albumin; Bovine Plasma Albumin; BSA

### STRUCTURE:

The molecular weight of BSA has frequently been cited as 66,120<sup>1</sup> or 66,267<sup>2</sup>, but it was revised in 1990 to 66,430<sup>3</sup>. All three values are based on amino acid sequence information available at the time of publication.

BSA is a single polypeptide chain consisting of about 583 amino acid residues and no carbohydrates. At pH 5-7 it contains 17 intrachain disulfide bridges and 1 sulfhydryl group.<sup>1,3</sup>

### PHYSICAL PROPERTIES:

Appearance: Powder - White to light tan <sup>4</sup> ;					
Solutions - Clear to slightly hazy and amber <sup>4</sup>					
pl in Water at 25°C: Endogenous Material <sup>5,6,7</sup> - 4.	7; 4.9;				
Fatty Acid Depleted <sup>8</sup> - 5.3					
pH of 1% Solution: <sup>1,4</sup> 5.2-7;					
Optical Rotation: <sup>1,9</sup> $[\alpha]_{259}$ : -61°; $[\alpha]_{264}$ : -63°					
Stokes Radius (r <sub>s</sub> ): <sup>10</sup> 3.48 nm					
Sedimentation constant, <sup>1</sup> S <sub>20,W</sub> X 10 <sup>13</sup>	4.5 (monomer), 6.7 (dimer)				
Diffusion constant, <sup>1</sup> $D_{20,W} \times 10^7$	5.9				
Partial specific volume, <sup>1</sup> V <sub>20</sub>	0.733				
Intrinsic viscosity, <sup>1</sup> η	0.0413				
Frictional ratio, <sup>1</sup> f/f <sub>o</sub>	1.30				
Overall dimensions, <sup>1</sup> Å	40 X 140				
Refractive index increment <sup>1</sup> (578 nm) X 10 <sup>-3</sup>	1.90				
Optical absorbance, <sup>1</sup> A <sup>1 gm/L</sup>	0.667				
Mean residue rotation, <sup>1</sup> $[m]_{233}$	8443				
Mean residue ellipticity <sup>1</sup>	21.1 [θ] <sub>209 nm</sub> ; 20.1 [θ] <sub>222 nm</sub>				
Estimated $\alpha$ -helix, <sup>1</sup> %	54				
Estimated β-form, <sup>1</sup> %	18				

#### **STABILITY / STORAGE AS SUPPLIED:**

If stored at 2-8°C, BSA powders and BSA solutions offered by Sigma are stable for a minimum of 2.5 years.<sup>4</sup>

## SOLUBILITY / SOLUTION STABILITY:

Albumins are readily soluble in water and can only be precipitated by high concentrations of neutral salts such as ammonium sulfate. Sigma tests the solubility of powdered BSA in deionized water at 40 mg/mL and obtains clear to very slightly hazy, faint yellow solutions. The solution stability of BSA is very good (especially if the solutions are stored as frozen aliquots). In fact, albumins are frequently used as stabilizers for other solubilized proteins (e.g., labile enzymes). However, albumin is readily coagulated by heat.<sup>11</sup> When heated to 50°C or above, albumin quite rapidly forms hydrophobic aggregates which do not revert to monomers upon cooling.<sup>4</sup> At somewhat lower temperatures aggregation is also expected to occur, but at relatively slower rates.

### **METHOD OF PREPARATION:**

- A. HISTORY:<sup>1,4</sup> Albumin is relatively simple to isolate and purify. One of the first methods of isolation involved extensive dialysis of serum against water; this process removed most globulins. A second procedure took advantage of the good solubility of albumin at low to moderate ammonium sulfate concentrations, and effected precipitation by lowering the pH. Electrophoretic isolation was also employed, as was affinity chromatography. None of these methods were applicable to large scale production.
- B. INITIAL ISOLATION: Initial isolation is by Heat Treatment or by Alcohol precipitation. Most commercial preparations are now prepared by Alcohol Precipitation a method developed by E. J. Cohn and his associates in the 1940's ("Fraction V" yields albumin with a purity of about 96%) or by Heat Treatment.<sup>12</sup>
- C. FURTHER PURIFICATION:<sup>1,4</sup> Additional removal of impurities can be accomplished by crystallization (a procedure which yields ≥99% pure albumin), preparative electrophoresis, ion exchange chromatography, affinity chromatography (e.g., ConA-agarose removes glycoproteins), heat treatment (removes globulins), low pH treatment, charcoal treatment, organic solvent precipitation (i.e., isooctane), and low temperature treatment.<sup>13</sup> Charcoal treatment and organic solvent precipitation remove fatty acids.<sup>13</sup>

#### **PRODUCT DESCRIPTION / USAGE:**<sup>14</sup>

Albumins are a group of acidic proteins which occur plentifully in the body fluids and tissues of mammals and in some plant seeds. Unlike globulins, albumins have comparatively low molecular weights, are soluble in water, are easily crystallized, and contain an excess of acidic amino acids. Serum and plasma albumin is carbohydrate-free and comprises 55-62% of the protein present.

#### **PRODUCT DESCRIPTION / USAGE: (continued)**

Albumin binds water, Ca<sup>2+</sup>, Na<sup>+</sup>, and K<sup>+</sup>. Due to a hydrophobic cleft, albumin binds fatty acids, bilirubin, hormones and drugs. The main biological function of albumin is to regulate the colloidal osmotic pressure of blood. Human and bovine albumins contain 16% nitrogen and are often used as standards in protein calibration studies. Albumin is used to solubilize lipids, and is also used as a blocking agent in Western blots or ELISA applications. Globulin free albumins are suitable for use in applications where no other proteins should be present (e.g., electrophoresis).

#### CHOOSING A PRODUCT:

Please refer to the table below for a complete description of each product. Based on customer input, literature reports and Sigma's own use, the following table lists product numbers which have successfully been used for specific applications. The list is not comprehensive, and product numbers not listed may often be substituted.

APPLICATION	PRODUCT NUMBER(S)
Antibody purification	A-2058
Binding and transport studies	A-4378, A-7030, A-0281, A-3675, A-3902, A-6003
Blood banking reagents	A-2153, A-4503, A-7888, A-3294, A-3912, A-7906, A-7030
Culture media (microbial)	A-2153, A-4503, A-3294, A-3912, A-7906, A-9430, A-7638, A-6003
Cell culture (general)	A-8806, A-9418
Electrophoresis (M.W. standard)	A-7517
ELISA (blocking reagent)	A-2153, A-4503, A-4378, A-7030, A-9430, A-3902
ELISA (non-specific binding)	A-3294
Enzyme systems	A-2153, A-4503, A-7888, A-3294, A-3912, A-4378, A-7906, A-7030, A-9430, A-7638, A-3675
Hapten carrier	A-7030, A-6003
Immunocytochemistry	A-9647, A-7906, A-6793
Immunohematology	A-2153, A-4503, A-7888, A-3294, A-3912, A-4378, A-7906, A-7030, A-0281, A-6003
Mitogenic assays	A-2058
Molecular biology	B-2518 <sup>15</sup> , B-8894 <sup>15</sup> , B-6917, B-8667, B-4287
Protein base or filler	A-2153, A-4503, A-3912, A-4378, A-7906, A-7030
Protein supplement (controls)	A-2153, A-4503, A-4378, A-7906, A-7030, A-3675
Protein standard (M.W., amino acids, nitrogen)	A-2153, A-4503, A-4378, A-7030
RIA systems	A-7888, A-4378, A-7030, A-3675, A-3902
Serology	A-4503, A-3912, A-4378, A-7906, A-7030, A-9430, A-3675

## **REFERENCES:**

- 1. *The Plasma Proteins: Structure, Function and Genetic Control*, 2nd ed., Frank W. Putnam, ed., Vol. 1, p. 141, 147, Academic Press, New York (1975).
- 2. Reed, R.G. et al., *Biochem. J.*, 191, 867 (1980).
- 3. Hirayama, K., *BBRC*, 173(2), 639 (1990).
- 4. Sigma data.
- 5. Dawson, R.M.C. et al., *Data for Biochemical Research*, 3rd ed., p. 381, Clarendon Press, Oxford (1993).
- 6. Malamud, D. and Drysdale, J.W., *Anal. Biochem.*, 86, 620 (1978).
- 7. Righetti, P.G. and Caravaggio, T., J. Chromatog., 127, 1 (1976).
- 8. Steinhardt, J. et al., *Biochem.*, 10(22), 630 (1971).
- 9. *CRC Handbook of Biochemistry: Selected Data for Molecular Biology*, H.A. Sober, ed., p. C-56, The Chemical Rubber Company, Cleveland (1968).
- 10. Axelsson, I., J. Chromatog., 152, 21 (1978).
- 11. Lewis, Sr., R.J. *Hawley's Condensed Chemical Dictionary*, 12th ed., p. 30, Van Nostrand Reinhold Co., New York (1993).
- 12. Cohn, E.J. et al., *J. Am. Chem. Soc.*, 68, 459 (1946).
- 13. Saifer, A. and Goldman, L. J. Lipid Res., 2(3), 268 (1961).
- 14. Scott, T. and Eagleson, M., *Concise Encyclopedia: Biochemistry*, 2nd ed., pp. 19-20, Walter de Gruyter, New York (1988).
- 15. These products are acetylated to inactivate nucleases commonly found in BSA, and are thus not listed in the table of unmodified BSA's on pp. 4-9 of this data sheet. Since tyrosines in the BSA are also derivatized, these preparations are not recommended for use as protein standards.

PRODUC T NUMBER	FURTHER PURIFICATION	PURITY BY AGAROSE GEL ELECTROPHORES IS	DESCRIPTION AND SPECIAL CHARACTERISTICS	PHYSICA L FORM	pH OF 1% (w/v) SOLUTION		
	INITIAL FRACTIONATION BY COLD ALCOHOL PRECIPITATION						
A2153		≥96%	Impurities: mostly globulins	Powder	~7		
A4503		≥96%	Impurities: mostly globulins	Powder	~5.2		
A7517		≥96%	Starting material: A-4503 Impurities: mostly globulins <b>Electrophoresis reagent</b> Suitable for use as a molecular weight marker (66 kDa) in SDS electrophoresis.	Powder	~5.2		
A9056		≥96%	Starting material: A-4503 Microbiologically tested	Powder	~5.2		
A3425		≥96%	Impurities: mostly globulins	Powder	~5.2		
A8551		≥96%	Impurities: mostly globulins BVD tested Cell culture tested	Powder	~5.2		
A7888		≥96%	<b>RIA grade</b> Suitable for use in insulin RIA procedures. May yield an insulin blank of ≤0.1 µunit/mg in certain procedures.	Powder	~5.2		
A4378	Crystallization	≥97%	1X Crystallized	Powder	~7		
A7511	Crystallization Charcoal	≥97%	Starting material: A-4378 Essentially fatty acid free (~0.005%)	Powder	~7		
A7638		≥99%	Starting material: A-4503 Essentially globulin free (<0.1 μg IgG/mg by HPLC)	Powder	~5.2		
A4161		≥99%	Starting material: A-7638 Essentially globulin free (<0.1 µg lgG/mg by HPLC) Cell culture tested	Powder	~5.2		
A9418		≥96%	Starting material: A-4503 Impurities: mostly globulins <b>Cell culture tested</b>	Powder	~5.2		

PRODUC T NUMBER	FURTHER PURIFICATION	PURITY BY AGAROSE GEL ELECTROPHORES IS	DESCRIPTION AND SPECIAL CHARACTERISTICS	PHYSICA L FORM	pH OF 1% (w/v) SOLUTION
A0281	Charcoal	≥99%	Starting material: A-7638 Essentially fatty acid free (~0.005%) Essentially globulin free (<0.1 µg IgG/mg by HPLC)	Powder	~5.2
A7417	Charcoal	≥99%	Starting material: A-0281 <sup>14</sup> <b>C-methylated</b> 5-50 μCi/mg Solution in 40 mM potassium phosphate buffer, pH 7.0, in serum bottle.	Solution	
A3902		≥98%	Starting material: A-7638 Vitamin $B_{12}$ and $B_{12}$ binding factor deficient For Vitamin $B_{12}$ assays 1 g contains <2.0 ng Vitamin $B_{12}$ and will bind <2.0 ng Vitamin $B_{12}$ . Essentially globulin free (<0.1 µg IgG/mg by HPLC)	Powder	~5.2
A6003	Charcoal	≥96%	Starting material: A-4503 Essentially fatty acid free (~0.005%)	Powder	~5.2
A3059	Heat treatment	~99%	Protease free (≤0.001 protease units/mg) Essentially -globulin free Prepared from pasteurized serum.	Powder	~7
A9543	Heat treatment Organic solvent precipitation	≥98%	Low endotoxin (≤0.1 ng/mg)	Powder	~7
A4919		≥98%	Low endotoxin (≤0.1 ng/mg) Cell culture tested	Powder	~7
A2934	Heat treatment Organic solvent precipitation	~99%	<b>Low endotoxin</b> (≤1 ng/mg) Essentially -globulin free Prepared from pasteurized serum.	Powder	~5.6
850-100		≥97%	Starting material: A-4378. Suitable for use in the determination of fibrin/fibrinogen degradation products in serum in the Staphylococcal clumping test per Sigma Procedure No. 850.	Powder	~7
A3156		≥ <b>99%</b>	<b>Cell culture tested</b> Sterilized by -irradiation	Powder	N/A

PRODUC T NUMBER	FURTHER PURIFICATION	PURITY BY AGAROSE GEL ELECTROPHORES IS	DESCRIPTION AND SPECIAL CHARACTERISTICS	PHYSICA L FORM	pH OF 1% (w/v) SOLUTION
A8412		≥96%	Starting material: A-4503 Impurities: mostly globulins <b>Cell culture tested</b> Prepared in DPBS. Sterile filtered. Endotoxin tested.	7.5% Solution	6.5-7.5
A9576		≥96%	Starting material: -4503 Impurities: mostly globulins <b>Cell culture tested</b> Prepared in DPBS. Sterile filtered. Endotoxin tested.	30% Solution	7.0-7.4
A8918		≥96%	Starting material: A-4503 Impurities: mostly globulins <b>Cell culture tested</b> Prepared in DPBS. Sterile filtered. Endotoxin tested.	35% Solution	4.9-5.7
A9205	Charcoal	≥96%	Starting material: A-6003 <b>Essentially fatty acid free</b> (~0.005%) Aseptically filled in 0.85% NaCl. No preservative added.	30% Solution	
A0336		≥98%	Starting material: A-9085 <b>Essentially IgG free</b> (≤25 ng IgG/mg by HPLC) Aseptically filled in 0.85% NaCl. No stabilizer added.	30% Solution	
A3424		≥96%	Starting material: A-4503. <b>Ultra-high avidity</b> Aseptically filled. No stabilizer added. Contains ~0.85% NaCl, as well as 0.1% sodium azide as preservative. Tested for avidity with known incomplete antibody (not saline agglutinable).	30% Solution	
A4628		≥96%	Starting material: A-4503 <b>Sterile filtered</b> Aseptically filled. Contains 0.70% NaCl, No preservative or stabilizer added.	5% Solution	

PRODUC T NUMBER	FURTHER PURIFICATION	PURITY BY AGAROSE GEL ELECTROPHORES IS	DESCRIPTION AND SPECIAL CHARACTERISTICS	PHYSICA L FORM	pH OF 1% (w/v) SOLUTION
		INITIAL FRACTION	IATION BY HEAT SHOCK		
A7906	Charcoal Dialysis	≥98%	Impurities: mostly globulins	Powder	~7
A6793	Charcoal Dialysis Deionization EtOH Treatment	≥98%	Impurities: mostly globulins	Powder	~7
A9647		≥98%	Starting Material: A9647	Powder	~7
A9085	Heat treatment Organic solvent precipitation	≥96%	Impurities: mostly globulins	Powder	~7
A3311		≥96%	Starting material: A-9647 Impurities: mostly globulins <b>Embryo tested</b>	Powder	~7
B6917		≥96%	Starting material: A9647 <b>Molecular Biology Grade</b> DNase, RNase, protease, alkaline phosphatase and peroxidase - none detected	Powder	~7
B8667		≥96%	Starting material: B6917 Molecular Biology Grade	20 mg/mL Solution	~7
A8022	Organic solvent precipitation	≥96%	Impurities: mostly globulins	Powder	~5.4
A3912	Charcoal Dialysis	≥98%	Impurities: mostly globulins	Powder	~5.2
A6918	Charcoal Dialysis	≥98%	Impurities: mostly globulins	Powder	~5.2
A7030	Charcoal	≥98%	<b>Essentially fatty acid free</b> (<0.02%) <b>Essentially -globulin free</b> Suitable as diluent in ELISA and RIA. Processed to reduce levels of $T_3$ , $T_4$ and insulin.	Powder	~7

PRODUC T NUMBER	FURTHER PURIFICATION	PURITY BY AGAROSE GEL ELECTROPHORES IS	DESCRIPTION AND SPECIAL CHARACTERISTICS	PHYSICA L FORM	pH OF 1% (w/v) SOLUTION
B4287	Charcoal	≥98%	Starting material: A-7030 Essentially fatty acid free (0.02%) Essentially -globulin free Molecular Biology Reagent Suitable as blocking agent is Southern blots. Not tested for DNase or RNase contamination; if a nuclease-free product is required, we recommend product B-2518: acetylated BSA.	Powder	~7
A3803		≥98%	Essentially fatty acid free (~0.005%) Suitable as diluent in ELISA.	Powder	~7
A3294	Charcoal	≥98%	Essentially protease free (≤0.005 protease units/mg) Impurities: mostly globulins	Powder	~7
A9430	Charcoal Dialysis	≥98%	Low endotoxin (≤1 ng/mg)	Powder	~7
A8806	Charcoal Dialysis	≥98%	Starting material: A-9430 Essentially fatty acid free (<0.005%) Low endotoxin (<0.1 ng/mg) Cell culture tested	Powder	~7
A7409		≥96%	Starting material: A-9647 Aseptically filled. Contains ~0.85% NaCl. No preservative added.	35% Solution	
A7534		≥96%	Starting material: A-9647 Aseptically filled. Contains ~0.85% NaCl, as well as 0.1% sodium azide as preservative.	35% Solution	
A8577	Charcoal	≥98%	Starting material: A-3294 <b>Essentially protease free</b> (<0.001 peroxidase units/mg) (<0.001 alk. phos. units/mg) Aseptically filled. Contains ~0.85% NaCl. No stabilizer or preservative added.	30% Solution	

PRODUC T NUMBER	FURTHER PURIFICATION	PURITY BY AGAROSE GEL ELECTROPHORES IS	DESCRIPTION AND SPECIAL CHARACTERISTICS	PHYSICA L FORM	pH OF 1% (w/v) SOLUTION
A8327		≥96%	Starting material: A-9647 Aseptically filled. Contains ~0.85% NaCl. No stabilizer or preservative added.	30% Solution	
A7284		≥96%	Starting material: A-9647 Aseptically filled. Contains ~0.85% NaCl, as well as 0.1% sodium azide as preservative. No stabilizer added.	30% Solution	
A1662		≥96%	Starting material: A-9647 Aseptically filled. Contains ~0.85% NaCl, as well as 0.1% sodium azide as preservative. Stabilized with octanoic acid (10-15 mg/g protein).	30% Solution	
A3299		≥96%	Starting material: A-9647 <b>High avidity</b> Aseptically filled. Contains ~0.85% NaCl, as well as 0.1% sodium azide as preservative. No stabilizer added. Tested for avidity with known incomplete antibody (not saline agglutinable).	30% Solution	
A3174		≥96%	Starting material: A-9647 <b>High avidity</b> Aseptically filled. Contains ~0.85% NaCl, as well as 0.1% sodium azide as preservative. Stabilized with octanoic acid (10-15 mg/g protein). Tested for avidity with known incomplete antibody (not saline agglutinable).	30% Solution	
A7034		≥96%	Starting material: A-9647 Aseptically filled. Contains ~0.85% NaCl, as well as 0.1% sodium azide as preservative.	22% Solution	
A7159		≥96%	Starting material: A-9647 Aseptically filled. No preservative added.	25% Solution	

PRODUC T NUMBER	FURTHER PURIFICATION	PURITY BY AGAROSE GEL ELECTROPHORES IS	DESCRIPTION AND SPECIAL CHARACTERISTICS	PHYSICA L FORM	pH OF 1% (w/v) SOLUTION
		INITIAL FRACTIONATI	ON BY SALT PRECIPITATION		
A9306	Chromatography	≥97%	Essentially globulin free Low endotoxin (≤0.1 ng/mg) Made from bovine plasma produced in New Zealand.	Powder	~7
A2058	Chromatography	≥97%	Essentially globulin free (<0.05% IgG by agarose gel electrophoresis) Low endotoxin (≤0.1 ng/mg) Cell culture tested	Powder	~7
A3675	Chromatography	≥98%	Low endotoxin (≤0.1 ng/mg)	Powder	~7
A1933	Chromatography	≥98%	Low endotoxin (≤0.1 ng/mg) Cell culture tested	Powder	~7

Sigma warrants that its products conform to the information contained in this and other Sigma-Aldrich publications. Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply. Please see reverse side of the invoice or packing slip.