

Status: Currently Official on 15-Feb-2025
 Official Date: Official as of 01-Jan-2018
 Document Type: NF Monographs
 DocId: GUID-E1DEB001-D148-40A2-B483-AD830CAC2083_3_en-US
 DOI: https://doi.org/10.31003/USPNF_M1496_03_01
 DOI Ref: o2yyd

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Alpha-Lactalbumin

$C_{626}H_{958}N_{162}O_{196}S_9$ 14178 CAS RN[®]: 9051-29-0.

DEFINITION

Alpha-Lactalbumin is a lyophilized or spray-dried powder of compact globular metalloprotein that may contain a single bound calcium ion and is capable of binding zinc and other metals. Alpha-Lactalbumin is isolated either from bovine milk or from whey, both of which should be from edible sources suitable for human use. All materials derived from bovine sources must originate from countries free of bovine spongiform encephalopathy. It contains alpha-lactalbumin at NLT 90.0% of the labeled total protein content. The remainder consists mostly of beta-lactoglobulin. It may contain suitable stabilizers.

IDENTIFICATION

• A. SDS-POLYACRYLAMIDE GEL ELECTROPHORESIS

Gel fixing solution: In a 1-L Pyrex bottle, thoroughly mix 500 mL of water, 400 mL of alcoholic TS, and 100 mL of glacial acetic acid.

Gel staining solution: Prepare a solution of Coomassie blue G-250 having a concentration of 0.25 g/L in a 10.0% (v/v) acetic acid solution.¹ Store at room temperature.

Destaining solution: 10.0% (v/v) acetic acid in water [NOTE—This solution may be stored at room temperature for up to 6 months from the date prepared.]

Sample buffer: Prepare a solution containing 200 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl), 2% (w/v) sodium dodecyl sulfate (SDS), 40% (v/v) glycerol, and 0.04% (w/v) Coomassie blue G-250. If necessary, adjust with hydrochloric acid or sodium hydroxide to a pH of 6.8.²

Running buffer: Prepare a solution containing 100 mM tris(hydroxymethyl)aminomethane, 100 mM *N*-tris(hydroxymethyl)methylglycine (tricine), and 0.1% (w/v) SDS in water. If necessary, adjust with hydrochloric acid or sodium hydroxide to a pH of 8.3.³ In a 400-mL beaker, thoroughly mix 35 mL of the solution so obtained (or 10× Tris/Tricine/SDS buffer)⁴ with 315 mL of water.

Molecular weight marker: Use a suitable molecular weight marker containing protein bands between 3.5 and 27 kDa.

Molecular weight standard solution: Transfer 16 µL of the *Sample buffer* into a 0.5-mL microcentrifuge tube. Pipet 4 µL of the *Molecular weight marker* into the microcentrifuge tube, and mix. Incubate the mixture in the closed microcentrifuge tube for 5 min at 95°. After incubation, allow the tube to stand for 5 min at room temperature. Centrifuge at 5000 rpm for 1 min.

Alpha-Lactalbumin standard stock solution: 1.0% (w/v) of [USP Alpha-Lactalbumin RS](#) in water in a 2-mL centrifuge tube.

Alpha-Lactalbumin standard working solution: Pipet 21 µL of the *Sample buffer* and 3 µL of the *Alpha-Lactalbumin standard stock solution* into a 0.5-mL microcentrifuge tube, and mix. Proceed as directed for *Molecular weight standard solution* beginning with “Incubate the mixture”.

Sample stock solution: 1.0% (w/v) of Alpha-Lactalbumin in water in a 2-mL centrifuge tube.

Sample solution: Pipet 21 µL of the *Sample buffer* and 3 µL of the *Sample stock solution* into a 0.5-mL microcentrifuge tube, and mix. Proceed as directed for *Molecular weight standard solution* beginning with “Incubate the mixture”.

SDS-PAGE gel and apparatus set-up: Following the manufacturer's instructions, assemble and fill a 16.5% Tris-Tricine Ready Gel⁵ in the Mini-Protean III Electrophoresis Module,⁶ or in an equivalent module. Add *Running buffer* appropriately to this apparatus.

Analysis

Gel loading: Load 10 µL of the *Molecular weight standard solution*, 2.5 µL of the *Alpha-Lactalbumin standard working solution*, and 2.5 µL of the *Sample solution*, respectively, into the 16.5% Tris-Tricine SDS-PAGE gel. [NOTE—The loaded samples contain approximately 3 µg of protein based on the sample weight.]

Running the gel: Set the voltage to 100 V, and run at a constant voltage. Run the gel until the tracking dye front is approximately 10 mm from the bottom of the gel (approximately 80–90 min).

Gel fixing: Remove the gel, transfer to a plastic container, and soak in the *Gel fixing solution* for 30 min on a shaking rack. Decant the *Gel fixing solution*. Rinse with water, and decant.

Gel staining: Pour approximately 100 mL of the *Gel staining solution* into the staining container. Place the gel into the staining container, and allow the stain to completely cover the gel. Place the staining container on an appropriate shaker, and stain the gel for 60–90 min with gentle shaking.

Destaining: Drain the *Gel staining solution* into an appropriate waste container, and add 100 mL of *Destaining solution* to the container to cover the gel. Place the container on an appropriate shaker, and shake with gentle agitation for 30 min. Discard the used *Destaining solution*, and repeat destaining as necessary. Repeat rinsing with *Destaining solution* three to four times at 30-min intervals or until the gel is destained to the desired clarity.

Acceptance criteria: The Alpha-Lactalbumin has one major band at 14 kDa, a minor band at 16 kDa, and a molecular weight that is similar to that of [USP Alpha-Lactalbumin RS](#).

- **B.** The retention time of the major peak for alpha-lactalbumin from the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the test for *Content of Alpha-Lactalbumin* in the Assay.

ASSAY

• CONTENT OF ALPHA-LACTALBUMIN

Mobile phase: Prepare a solution of 0.02 M Tris-HCl, 0.5% SDS, and 0.1 N sodium chloride. Adjust the pH of the solution to 5.95 ± 0.05 . Pass this solution through a filter having a 0.5- μ m or finer porosity, and degas.

Standard solution: 1.0 mg/mL of [USP Alpha-Lactalbumin RS](#) in *Mobile phase*. [NOTE—Prepare it immediately before use.]

System suitability solution: 0.5 mg/mL of [USP Alpha-Lactalbumin RS](#) and 0.5 mg/mL of beta-lactoglobulin in *Mobile phase*

Sample solution: 1.0 mg/mL of Alpha-Lactalbumin in *Mobile phase*

Chromatographic system

(See [Chromatography \(621\)](#), System Suitability.)

Mode: LC

Detector: UV 280 nm

Column: 7.8-mm $\times$ 30-cm analytical column, packing L33

[NOTE—Equilibrate the column for approximately 90 min at 0.6 mL/min of *Mobile phase* or until a stable baseline is achieved.]

Flow rate: 0.6 mL/min

Injection size: 20 μ L

System suitability

Sample: *System suitability solution*

[NOTE—The relative retention times for beta-lactoglobulin and alpha-lactalbumin are 0.91 and 1.00, respectively.]

Suitability requirements

Resolution: NLT 1.65 between beta-lactoglobulin and alpha-lactalbumin

Tailing factor: Not greater than 1.1 for the alpha-lactalbumin peak

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the purity of Alpha-Lactalbumin as a percentage of total protein:

$$\text{Result} = (r_U/r_S) \times [C_S/(C_U \times P)] \times 100$$

r_U = peak response for alpha-lactalbumin from the *Sample solution*

r_S = peak response for alpha-lactalbumin from the *Standard solution*

C_S = concentration of [USP Alpha-Lactalbumin RS](#) in the *Standard solution* (mg/mL)

C_U = concentration of Alpha-Lactalbumin in the *Sample solution* (mg/mL)

P = percentage of total protein content

Acceptance criteria: Content of alpha-lactalbumin is NLT 90.0% of the labeled total protein content.

• TOTAL PROTEIN CONTENT

Sample: 250 mg of Alpha-Lactalbumin

Analysis: Combust the *Sample* in the presence of pure oxygen (99.9%) in an airtight oven at 950° with a suitable nitrogen analyzer. The components such as carbon dioxide, sulfur dioxide, and moisture are absorbed by various in-line chemical filters. All nitrogenous matter is converted into nitrogen in the presence of catalytic converters. The weight percent of nitrogen is measured by a thermal conductivity detector. Blank the system by analyzing a suitable nitrogen blank material, such as powdered cellulose, and obtaining a zero reading.

Calibrate and qualify the system by using EDTA. The relative standard deviation for replicate runs is NMT 0.5%. Calculate the weight percent of total protein content in Alpha-Lactalbumin by multiplying the percentage of nitrogen found by 6.23.

Acceptance criteria: Total protein content is NLT 90.0%.

OTHER COMPONENTS

• LIMIT OF BETA-LACTOGLOBULIN

Mobile phase, System suitability solution, Sample solution, Chromatographic system, and System suitability: Prepare as directed in the test for *Content of Alpha-Lactalbumin*.

Standard solution: 1.0 mg/mL of beta lactoglobulin in *Mobile phase*. [NOTE—Prepare it immediately before use.]

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of beta-lactoglobulin as a percentage of total protein:

$$\text{Result} = (r_U/r_S) \times [C_S/(C_U \times P)] \times 100$$

r_U = peak response for beta-lactoglobulin from the *Sample solution*

r_S = peak response for beta-lactoglobulin from the *Standard solution*

C_S = concentration of beta-lactoglobulin in the *Standard solution* (mg/mL)

C_U = concentration of Alpha-Lactalbumin in the *Sample solution* (mg/mL)

P = percentage of total protein content

Acceptance criteria: NMT 6.5%, calculated on the total protein basis

• CONTENT OF CALCIUM

Standard stock solution: Dissolve 1.249 g of calcium carbonate in 270 mL of 3 N hydrochloric acid (dilute 250 mL of hydrochloric acid with water to 1000 mL) in a 1000-mL volumetric flask. Dilute with water to volume, and mix. Dilute 50 mL of the solution so obtained to 1000 mL. The *Standard stock solution* contains 25 µg/mL of calcium.⁷

Lanthanum chloride solution: Weigh 11.7 g (± 100 mg) of lanthanum oxide, and transfer to a 1000-mL volumetric flask. Add enough water to wet the powder, and then slowly add 50 mL of hydrochloric acid. [CAUTION—Exothermic reaction.] Let the test specimen dissolve, dilute with water to volume, and mix. This solution contains 1% (w/v) of lanthanum and is stable for up to 6 months when stored at room temperature.

Blank solution: 10-fold dilution of *Lanthanum chloride solution*

Working standard solutions: To five identical 25-mL volumetric flasks add 0, 5, 10, 15, and 20 mL, respectively, of *Standard stock solution*. Add 2.5 mL of *Lanthanum chloride solution*, and dilute with water to volume. The *Working standard solutions* contain 0, 5, 10, 15, and 20 µg/mL of calcium, each containing 0.1% (w/v) of lanthanum.

Sample solution: Transfer 1.0 g of Alpha-Lactalbumin to a 100-mL volumetric flask, add 10 mL of *Lanthanum chloride solution*, and dilute with water to volume.

Spectrometric conditions

(See [Atomic Absorption Spectroscopy \(852\)](#).)

Mode: Atomic absorption spectrophotometry

Analytical wavelength: 422.7 nm

Lamp: Calcium hollow-cathode lamp

Flame: Reduced air–acetylene

Analysis

Samples: *Working standard solutions* and *Sample solution*

Concomitantly determine the absorbances of the *Samples* using the *Blank solution*. [NOTE—Optimize flame parameters in accordance with the instrument manufacturer's instructions.]

Plot the absorbances of the *Working standard solutions* versus the concentration, in µg/mL, of calcium, and draw the straight line best fitting the five plotted points. From the graph so obtained, determine the concentration, C, in µg/mL, of calcium in the *Sample solution*.

Calculate the quantity of calcium (Ca), in mg, in each g of Alpha-Lactalbumin taken:

$$\text{Result} = (V \times C)/W \times F \times 100$$

V = volume of the *Sample solution*, 100 mL

C = as determined above