Status: Currently Official on 17-Feb-2025
Official Date: Official as of 01-Jan-2018
Document Type: NF Monographs
DocId: GUID-2FFA5A3C-B92C-44E6-B3A2-4E77C26AEB56_3_en-US
DOI: https://doi.org/10.31003/USPNF_M89500_03_01
DOI Ref: oy4z2

Zein

CAS RN®: 9010-66-6.

DEFINITION

Zein is a prolamine derived from corn [*Zea mays Linné* (Fam. Gramineae)].

IDENTIFICATION

- **A.**

  Sample solution: Dissolve 0.1 g in 10 mL of 0.1 N sodium hydroxide.

  Analysis: To the *Sample solution* add a few drops of cupric sulfate TS. Warm in a water bath.

  Acceptance criteria: A purple color develops.

- **B.**

  Sample solution: In a test tube add 1 mL of nitric acid to 25 mg of Zein.

  Analysis: Agitate the *Sample solution* vigorously.

  Acceptance criteria: The solution becomes light yellow. Further addition of about 10 mL of 6 N ammonium hydroxide produces an orange color.

- **C. Identification of alpha-Zein by SDS-Polyacrylamide Gel Electrophoresis**

  Solvent: 55% Isopropyl alcohol with 2% beta mercaptoethanol

  Sample loading buffer:[1] 0.5 M tris hydrochloride pH 6.8, 20% glycerin, 4% sodium dodecyl sulfate (SDS), and 0.005% bromophenol blue

  Gel running buffer stock solution:[2] 0.25 M tris base pH 8.6, 1.92 M glycine, and 1.0% SDS

  Gel running buffer: *Gel running buffer stock solution* and water (1:9)

  Gel staining solution: A suitable Coomassie blue-based solution[3]

  Molecular weight marker: Use a suitable molecular weight marker[4] containing protein bands at 10–190 kDa. [Note—A molecular weight marker with protein bands at 10–100 KDa can also be used.]

  Molecular weight standard solution: Dilute the *Molecular weight marker* with the *Sample loading buffer* (1:1). Incubate the mixture in a closed microcentrifuge tube at 95° for 10 min. After incubation, allow the tube to flash-cool on ice. Place the tube in a microcentrifuge, spin at a top speed for a few seconds, and allow to stop on its own to collect any condensation on the sides and top of the tube.

  Independent standard stock solution: 10 mg/mL of β-lactoglobulin A in water

  Independent standard working solution: 1 mg/mL of β-lactoglobulin A in water

  Independent standard running solution: To the final 1 volume of *Independent standard running solution*, add 0.1 volume of *Independent standard working solution*, 0.5 volume of *Sample loading buffer*, 0.1 volume of β-mercaptoethanol, and sufficient water to obtain 0.1 mg/mL of β-lactoglobulin A and 10% of β-mercaptoethanol.

  Sample stock solution: 10 mg/mL of Zein in *Solvent*. Mix on a vortex mixer until the sample is fully dissolved. Centrifuge at 10,000–12,000 rpm in a microcentrifuge with a fixed rotor for 10 min to pellet any undissolved material.

  Sample solution: Dilute the *Sample stock solution* with the *Sample loading buffer* (1:1). Incubate the mixture in a closed microcentrifuge tube for 10 min at 95°. After incubation, allow the tube to flash-cool on ice. Place the tube in a microcentrifuge, spin at a top speed for a few seconds, and allow to stop on its own to collect any condensation on the sides and top of the tube. Dilute the solution so obtained with a mixed solution of *Sample loading buffer* and water (1:1) to 5 folds.

  Electrophoretic system

  SDS-PAGE gel and apparatus setup: Following the manufacturer's instructions, assemble and fill a precast 16% Tris-Glycine gel[5] in an appropriate electrophoresis module.

  Running buffer: *Gel running buffer*

  Voltage: 100 V

Run time: 2.5 h or until the upper dye front is at the bottom of the gel. [NOTE—The total run time may need to be altered, depending on the molecular weight standards as well on as laboratory equipment variability, because the dye front may co-migrate with or close to the lowest bands of the set.]

Analysis

Samples: *Molecular weight standard solution*, *Independent standard running solution*, and *Sample solution*

Gel loading: Load 25 µL of the *Molecular weight standard solution*. Load a volume of the *Independent standard running solution*, equal to 2 µg of β-lactoglobulin A. Load a volume of the *Sample solution*, equal to approximately 5 µg of calculated Zein. [NOTE—If the 5 µg loading amount is inadequate for the test, the 10 µg loading amount may be used. Loading a lesser quantity on the gel results in an overall higher molecular weight determination due to sharper bands. The actual amount of Zein extracted from the starting material cannot be quantified. Therefore, an estimated amount is derived.]

Gel staining: After electrophoresis, carefully remove the gel from the plates. Rinse the gel three times with water. Stain the gel by following the manufacturer's directions for the stain used.

Destaining: After staining as directed by the manufacturer, destain as directed by the manufacturer.

Gel scan procedure: Set up a gel scanner according to the manufacturer's instructions. Place the gel in the detector, and obtain a single image of all loaded lines of the gel.

Acceptance criteria: Zein has two major bands for α-zein at 15–26 kDa.

IMPURITIES

• **Residue on Ignition ⟨281⟩:** NMT 2.0%, using an ignition temperature of 800 ± 25°

• **Limit of Hexane-Soluble Matter**

Sample: 15 g of Zein

Solvent: Alcohol and water (17:3, w/w)

Analysis: Dissolve the *Sample* in 150 mL of *Solvent*. Stir the mixture, using a magnetic stirrer, and heat the solution to 30°. Once the *Sample* is dissolved, transfer the solution to a 500-mL separatory funnel.

Add 60 mL of *n*-hexane. Shake the mixture, and allow the phases to separate. Discharge the bottom layer (alcohol) to a beaker, and transfer the top layer (hexane) to a first 500-mL flask. Weigh the first 500-mL flask, and record the weight. Pour the bottom layer of alcohol back into the separatory funnel. Repeat this step four more times.

After the five 60-mL hexane solutions have been added to the first 500-mL flask, attach it to a rotary evaporator to distill the hexane. Collect the hexane in a second 500-mL flask.

The first 500-mL flask contains a yellow to reddish oil. Record the weight of the flask containing this oil.

Calculate the percentage of hexane-soluble matter in the portion of Zein taken:

$$\text{Result} = [(W_T - W_F)/W] \times 100$$

W_T = weight of the flask (g)

W_F = weight of the first 500-mL flask (g)

W = weight of the *Sample* (g)

Acceptance criteria

For Zein from normal dent corn: NMT 12.5% for hexane-soluble matter

For Zein from waxy corn: NMT 16.0% for hexane-soluble matter

SPECIFIC TESTS

• **Loss on Drying ⟨731⟩**

Analysis: Dry at 105° for 2 h.

Acceptance criteria: NMT 8.0%

• **Microbial Enumeration Tests ⟨61⟩** and **Tests for Specified Microorganisms ⟨62⟩:** The total bacterial count does not exceed 10^3 cfu/g, and the tests for *Salmonella* species and *Escherichia coli* are negative.

• **Protein Content**

Analysis: Proceed as directed in *Nitrogen Determination ⟨461⟩, Method I*. Calculate the weight percentage of the protein content in Zein by multiplying the percentage of nitrogen found by 6.25.

Acceptance criteria: 81.9%–100.0% on the dried basis

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in well-closed containers, and store at room temperature.

• **Labeling:** Label it to indicate the corn source from which it is derived.

[1] Available from Invitrogen (Life Technologies) as Tris-Glycine SDS Sample Buffer (2X), catalog number LC2676.

[2] Available from Invitrogen as Tris-Glycine SDS Running Buffer (10X), catalog number LC2675.

[3] Available from Invitrogen as SimplyBlue Stain, catalog number LC6065.

[4] Available from Invitrogen as BenchMark Prestained Protein Ladder, catalog number 1074810.

[5] Available from Invitrogen, catalog number EC6495. However, these are readily available from several other manufacturers.

Auxiliary Information - Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
ZEIN	Documentary Standards Support	CE2020 Complex Excipients
REFERENCE STANDARD SUPPORT	RS Technical Services RSTECH@usp.org	CE2020 Complex Excipients

Chromatographic Database Information: Chromatographic Database

Most Recently Appeared In:
Pharmacopeial Forum: Volume No. PF 40(5)

Current DocID: GUID-2FFA5A3C-B92C-44E6-B3A2-4E77C26AEB56_3_en-US
Previous DocID: GUID-2FFA5A3C-B92C-44E6-B3A2-4E77C26AEB56_1_en-US
DOI: https://doi.org/10.31003/USPNF_M89500_03_01
DOI ref: oy4z2