

Operation Manual

Shodex STANDARD P-82

(Please read this manual carefully to achieve accurate and consistent molecular weight information for a long time)

Important Handling Instructions

Caution!

- Please consult the Safety Data Sheet (SDS) of reagents and solvents used with the column and understand their proper handling methods to prevent potential health hazards or death from occurring.
- Please wear appropriate personal protective equipment such as lab goggles and gloves when handling organic solvents and acid and alkaline reagents. Avoid any direct physical contact to prevent chemical injuries.

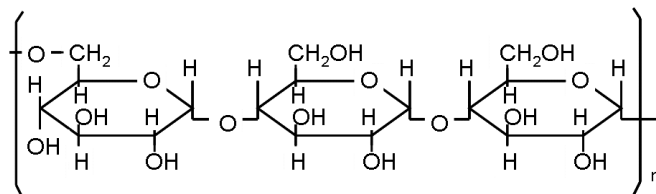
Before Using the Standard

- (1) Please visually inspect the package and outside of the reagent bottles for any damage.
- (2) Please check if product name and lot number written on the package, reagent bottle adhesive labels, and enclosed INSPECTION CERTIFICATE are matching and correct.
- (3) Please download INSPECTION CERTIFICATE for the purchased product. INSPECTION CERTIFICATE can be downloaded from Shodex website (<https://www.shodex.com/download/>). You will be asked to enter the lot number.

1. Introduction

Thank you for purchasing the Shodex product. Shodex STANDARD P-82 is pullulan standards which are neutral polysaccharides with a non-branched linear structure containing repeating α -1,6 linked maltotriose molecules. They dissolve easily in water, and rarely associate nor crystallize. They also have narrow molecular weight distributions and are less likely to adsorb to the column packing material. Therefore, the standards are suitable for preparing calibration curves required in relative molecular weight distribution analyses by aqueous size exclusion chromatography (SEC).

2. Structural Formula



3. Product Information

Product Code	Product Name	Contents	Molecular Weight (Mp) Range
F8400000	STANDARD P-82	0.2 g x 8 kinds	approx. 5,000 - 800,000

(Note) Molecular weights (Mp, Mw/Mn) of a standard kit may vary depending on production lot.

4. How to Use Standards

4.1 Sample Solution Preparation

- (1) Viscosity of high molecular weight compound is largely influenced by its molecular weight and concentration. Samples with high viscosity cause peak broadening and elution delay, and this makes it difficult to obtain their accurate molecular weight distributions. In general, the larger the molecular weight of the compound, the higher its viscosity becomes. To suppress the influence from high viscosity, it is recommended to lower the sample concentration. Please use the below table as a reference when preparing samples for molecular weight distribution analyses.

Molecular Weight Range	Optimal Concentration (w/v)
5,000 - 25,000	≤ 0.5 %
25,000 - 200,000	≤ 0.25 %
200,000 - 1,000,000	≤ 0.1 %

- (2) Add solvent to standard and let it stand to swell. The time required for swelling and dissolution depends on molecular weight of a standard. Lower-molecular-weight standards swell and dissolve in a few hours. However, higher-molecular-weight standards require a longer time. Leave them in a refrigerator for half a day to a day until they fully swell.
- (3) Once standard is fully swollen, gently stir the sample solution to let it completely dissolve and homogenize.
- (4) Filter the prepared sample solutions using disposable 0.45- μ m filters.
- (5) Sample solutions should be stored in a refrigerator (about 4 °C recommended) to prevent it from bacterial growth. Before storing the sample, adjust the sample pH between 5 and 7.
- (6) Use refrigerated sample solutions within 1 week.

Attention!

- Ultra-sonication may cause shear degradation if used to dissolve standards, and thus not recommended.
- Bacteria can decompose standard samples. Moreover, the presence of bacteria in samples may damage the column if injected.
- The standards dissolved in sodium nitrate aqueous solution may decompose even when stored in a refrigerator. Please prepare fresh sample solutions at each analysis if sodium nitrate aqueous solution is used to dissolve the standards.

Note

- Please refer below article for physical properties of pullulan solutions.
T. Kato, T. Okamoto, T. Tokuya, A. Takahashi. "Solution properties and chain flexibility of pullulan in aqueous solution" Biopolymers 21(8), 1982, 1623-1633

4.2 Drying

Standards contain a small amount of water. Dry standards before sample solution preparation to obtain desired concentrations with higher accuracy. Standards contain a small amount of water. If concentration with an exact accuracy is required, dry the standard before sample solution preparation. Below are example drying methods.

P-5 to P-100: Vacuum dry at 90 °C for 6 hours or longer

P-200 to P-800: Leave in a desiccator containing phosphorus pentoxide

Attention!

- Higher-molecular-weight standard samples (P-200 and higher) may partially decompose when heated.

4.3 Calibration Curve

Analyze standard samples under the same analysis conditions as target samples. Prepare a calibration curve for measuring molecular weight distribution using retention times of each standard sample and their peak top molecular weight (Mp) values stated on the INSPECTION CERTIFICATE.

4.4 Detection

Pullulan has a very weak UV absorbance, thus a UV detectors is not suitable. Generally, a RI detector is used.

5. Storage

Store undissolved standards in a desiccator or an airtight container and refrigerate (about 4 °C recommended). Do not open reagent bottles immediately after taking out from the refrigerator. Wait opening until the bottle temperature reaches the room temperature.

Attention!

- Presence of bacteria or mold in the air and/or on equipment may cause decomposition of polysaccharides.
- A sign of bacterial decomposition is discoloration. If such an abnormality was observed, do not use the standard.

6. Expiration Date

Expiration date of an unopened standard is 2 years from the inspection date.

Note

- The inspection date is stated on the INSPECTION CERTIFICATE.

Please refer to the Shodex website (<https://www.shodex.com/>) for product details and their applications. For additional assistance, contact the distributor from whom you purchased the column or contact your regional Shodex support office (https://www.shodex.com/en/support_office/list).