Operation Manual
Shodex™ STANDARD P-82, STD P series
(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 serial number written on the package, reagent bottle adhesive labels, and enclosed INSPECTION CERTIFICATE are matching and correct.

1. Introduction
Thank you for purchasing the Shodex™ product. Shodex™ STANDARD P-82 and STD P series are 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

![Structural Formula Image]

3. Product Lineup

Standard Kit

<table>
<thead>
<tr>
<th>Product Code</th>
<th>Product Name</th>
<th>Contents</th>
<th>Molecular Weight (Mp) Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>F8400000</td>
<td>STANDARD P-82</td>
<td>0.2 g x 8 kinds</td>
<td>approx. 5,000 - 800,000</td>
</tr>
</tbody>
</table>

Single Standards

<table>
<thead>
<tr>
<th>Product Code</th>
<th>Product Name</th>
<th>Contents</th>
<th>Molecular Weight (Mp) Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>F8400800</td>
<td>STD P-800</td>
<td>0.5 g</td>
<td>approx. 800,000</td>
</tr>
<tr>
<td>F8400400</td>
<td>STD P-400</td>
<td>0.5 g</td>
<td>approx. 400,000</td>
</tr>
<tr>
<td>F8400200</td>
<td>STD P-200</td>
<td>0.5 g</td>
<td>approx. 200,000</td>
</tr>
<tr>
<td>F8400100</td>
<td>STD P-100</td>
<td>0.5 g</td>
<td>approx. 100,000</td>
</tr>
<tr>
<td>F8400050</td>
<td>STD P-50</td>
<td>0.5 g</td>
<td>approx. 50,000</td>
</tr>
<tr>
<td>F8400020</td>
<td>STD P-20</td>
<td>0.5 g</td>
<td>approx. 20,000</td>
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<tr>
<td>F8400010</td>
<td>STD P-10</td>
<td>0.5 g</td>
<td>approx. 10,000</td>
</tr>
<tr>
<td>F8400005</td>
<td>STD P-5</td>
<td>0.5 g</td>
<td>approx. 5,000</td>
</tr>
</tbody>
</table>

(Note) Molecular weights (Mp, Mw/Mn) of a standard kit or each single type may vary depending on production lot.
4. How to Use Standards

4.1 Sample Solution Preparation
(1) Recommended standard sample concentration is 0.1 - 0.5 % (w/v).
(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-μm 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.

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.

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/).
7. Warranty (Ver. 4STD)

(1) Showa Denko K. K. warrants that the Shodex™ product, at the time of delivery to the user, will conform to the specification of the attached INSPECTION CERTIFICATE, if the Shodex product is used in accordance with the attached operating manual. The foregoing warranty is exclusive and is in lieu of all other warranties with respect to the Shodex product, whether written, oral, implied, statutory or otherwise. No warranties by Showa Denko K. K. are implied or otherwise created, including, but not limited to, the warranty of merchantability and fitness for particular purposes.

(2) Any claim of nonconformity to the specification must be notified to Showa Denko K.K. within ten (10) days after delivery to the user. User’s exclusive remedy and Showa Denko K.K.’s exclusive liability for such claim are limited to the replacement of the Shodex product in question. In no event is Showa Denko K.K. liable for any indirect, incidental or consequential damage arising out of in connection with the Shodex product, whether or not such damage is allegedly based on breach of warranty, negligence or otherwise.

(3) No warranty is made in any of the following cases:
3-1 If the Shodex product is not used in accordance with the operating manual
3-2 If the Shodex product is remodeled by anyone other than person or firm designated by Showa Denko K.K.
3-3 If the Shodex product is disposed of
3-4 If the Shodex product is resold by the user without giving prior written notice to Showa Denko K.K.
3-5 If the performance of the Shodex product is not conform to the specification of the attached INSPECTION CERTIFICATE due to any of the reasons below:
   a. Computer virus
   b. Impurities contained in the sample, reagent, gas air or cooling water provided by the user
   c. Breakdown or malfunction of equipment, apparatus or component used in combination with the Shodex product
   d. Force majeure such as fire, earthquake, flood, other natural disaster, crime, riot, act of terrorism, war or radioactive contamination

(4) In no event is Showa Denko K.K. liable for
4-1 the results of analysis or preparations using the Shodex product or any portion of the same, including, but not limited to, the reliability, accuracy, efficacy and safety of said results, and
4-2 the occupational hazard in the use of the Shodex product, whether or not such use is made in accordance with the attached Conditions for use.

(5) The Shodex product is for laboratory use only. It must not be used for clinical diagnosis. Showa Denko K.K. is not liable for any use of the Shodex product except laboratory use.

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Shodex, AFpak, Asahipak, AXpak, CLNpak, CXpak, HILICpak, MSpak, ODP, OHpak, ORpak, RSpak, SUGAR, USPpak.

Products described in the operation manual are not intended for medical use or medical applications.