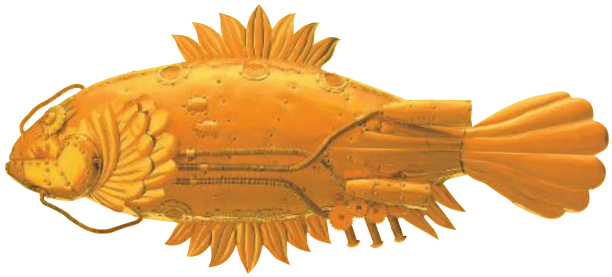
Shodex™



HPLC Columns

MANUAL

STANDARD
Pullulan P-82

SHOWA DENKO EUROPE

Columns manufactured by Showa Denko K.K Japan
Made in Japan

Shodex HPLC Columns
Europe, Middle East, Africa, Russia

For technical support please use contact details shown below:

SHOWA DENKO EUROPE GmbH
Shodex Business
Konrad-Zuse-Platz 4
81829 Munich, Germany

E-mail: support@shodex.de
Phone: +49 (0)89 93 99 62 37
www.shodex.de

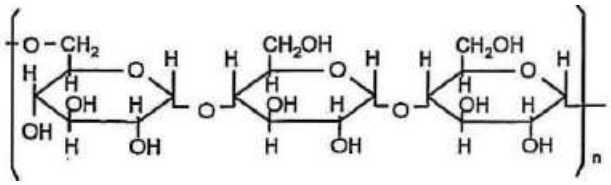
Operation Manual

Shodex™ STANDARD Pullulan P-82

(Please read this manual carefully before using the column to ensure performance and life.)

1. Introduction

Shodex STANDARD P-82 is a calibration standard for aqueous size exclusion chromatography. Pullulan is one of polysaccharides and its chemical structural formula is mentioned below. Units of maltotriose are bonded with α-1,6 connection, and its chains are linear without branched chains.



The sample solution of this standard is easy to prepare and easy to handle, because it is stable without agglomeration, crystallization or electrical-charge, and moreover pullulan is not adsorbed to column packing materials. This is good as molecular weight standard to m11ke calibration for Size Exclusion Chromatography (SEC).

2. Instructions in handling <Important>

Warning * Take notice of keeping instructions about the solvents and· the reagents used with the standard not to occur problems related to losing your health or leaking.

Caution! * Degradation of standard may happen under unsuitable storage condition. A standard contaminated by bacteria is a cause of column troubles. Use and store the standard in accordance with this operation manual. See the clause "Notice" about the permissible range.

3. Products

STANDARD P-82 kit is consisted of 8 kinds of different molecular weight with 200mg each. Please refer "certificate of analysis" regarding information of molecular weight. There is reference paper regarding property of material (1).

In order to make calibration curve, plot Mp and retention time which is used the same analysis condition with the target sample analysis. Molecular weight of unknown sample can be estimated as conversion of pullulan molecular weight using the calibration curve.

4. Notice

4-1. Storage of powder

Polysaccharides are degradated by bacteria or fungus existed in air or vessels generally. Please put the products into a desiccator or a sealed case and store in a refrigerator (around 4°C is recommended). Wait till the standard is warmed to room temperature, then open and use the standard.

It is only as a guide but rough life time is 2 years after purchasing for unopened products. Opened product is recommended to be used within 1 year after opening, please seal well and store in dark and cool place like a refrigerator. Pullulan is stable material itself but easy-to-degrade ted by bacteria. So dispose it when you see colored.

4-2. Preparing standard solution

Add distilled water to specified amount of standard and wait the sample swelled well. Stir it mildly, and check the sample is solved uniformly. It takes longer time to swell and solve with higher molecular weight standard. Keeping one day in a refrigerator is recommended to solve for P-800.

4-3. Storage of solution

Take care of preventing contamination of bacteria. Keep the solution in a refrigerator when the solution is not used. Do not store solution· without pH 5"'7 solvent (stronger acid or basic). And a standard contaminated bacteria is a cause of column troubles. Stored in a refrigerator within one week is recommended.

4-4. Drying

Standard contains a small amount of moisture. When dried standard is needed, keep it in vacuum dryer at 90°C more than 6 hours. Regarding P-200, P-400 and P-800, there is fear of heat digestion by vacuum dryer, so keep them in a desiccator with phosphorus pentoxide.

5. Preparing standard solution

Measure out 0.5 to 1.0 mg and add solution (distilled water, salt water or buffer) to 0.1~0.5% solution. Wait till the standard swells well. Low molecular weight standard swells in a few hours, but higher molecular weight standard is recommended to be waited in a half day to one day in a refrigerator. Stir it mildly after swelling well, and filtrate it with 0.45 μm filter, then use it as a standard sample.

6. Detection

Pullulan is polysaccharide, so general detection methods of saccharides are applied. Reflective index detector (RI) is generally used, but UV cannot be applied because there is not UV absorbance in saccharides.

7. Warranty

1) Showa Denko K. K. warrants that the Shodex Column, at the time of delivery to the user, will conform to the specification of the attached Certificate of Analysis, if the Shodex Column is used in accordance with the operating manual. The foregoing warranty is exclusive and is in lieu of all other warranties with respect to the Shodex Column, 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 inconformity 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 Column 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 Instrument, 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:

(1) If the Shodex Column is not used in accordance with the operating manual.

(2) If the Shodex Column is remodeled by anyone other than person or firm designated by Showa Denko K.K.

(3) If the Shodex Column is resold by the user without giving prior written notice to Showa Denko K.K.

(4) If the performance of the Shodex Column is not conform to the specification of the attached Certificate of Analysis 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 Column

d) Force majeure such as fire, earthquake, flood, other natural disaster, rime, riot, act of terrorism, war or radioactive contamination

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

5) The Shodex instrument 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 Instrument except laboratory use.

8. Reference

(1) T.Kato, T.Okamoto, T.Tokuya, A.Takahashi, Biopolymers 21, 1623 (1982)

- Contact to http:/ /www.shodex.com
- Manufactured by SHOWA DENKO K.K. Shodex (Separation & HPLC) Group

5-1, Ogimachi, Kawasaki-ku, Kawasaki, Kanagawa 21D-0867 JAPAN