Product specification

Product number:	**GL-006**
Product name:	**PA-IL (LecA) lectin produced recombinantly in *E. coli*, unconjugated**
Form:	Lyophilized powder
Unit size:	1 mg, 5 mg, 10 mg
Sugar specificity:	D-galactose (see the detailed information below)
Protein sequence:	AWKGEVLANNEAGQVTSIIYNPGDVITIVAAGWASYGPTQKWGPQGDREHPDQGLICHDAFCG ALVMKIGNSGTIPVNTGLFRWVAPNNVQGAITLIYNDVPGTYGNNSGSFSVNIGKDQS
Storage conditions:	For long-term storage, keep the freeze-dried lectin at -20 °C. After dissolving, store the sample in the fridge.

This product is for R&D use only. Not for human or animal use.

Basic information:

Name:	PA-IL (LecA, PA-I)	Molecular weight (monomer):	12762.2 Da
Organism:	*Pseudomonas aeruginosa*	Extinction coefficient:	27960 M^{-1} cm^{-1}
Expression host:	*Escherichia coli*	Oligomeric state:	tetramer
Tags:	no		

Carbohydrate specificity:

PA-IL (LecA, formerly PA-I) lectin is specific towards carbohydrates containing terminal α-galactosides (bound via α1-3, α1-4, and α1-6 linkages), including αGal1–4Gal motif, typical for Gb3/Pk and P1 antigens, and αGal1-3Gal motif, present in human blood group B antigen [1-2]. Despite certain information in the literature, our data demonstrate that PA-IL binds β-galactosides in a solution.

Ion dependency: Ca^{2+} Glycan array data available at functionalglycomics.org

Stability:

Stable in a variety of buffers with pH 4.5 - 9.5. Since the presence of calcium ions in the binding site is required for lectin activity, adding calcium ions (0.1-0.5 mM $CaCl_2$) to the working buffer is recommended. Avoid EDTA and other chelating agents as they lead to removing Ca^{2+} ions from the binding site and losing lectin activity. After reconstitution in neutral pH buffers, the protein should be stable in the fridge for weeks. Adding sodium azide (0.02%) is recommended to avoid microbial growth.

T_m = 72 °C (DSC, 20 mM Tris, 150 mM NaCl, 0.1 mM $CaCl_2$, pH 7.5)

Applications and biological effects:

PA-IL can be used in various applications, including lectin blotting, fluorescence microscopy, flow cytometry, and lectin histochemistry. It is also used in commercial lectin microarrays to study glycosylations of proteins and cells.

PA-IL lectin inhibits ciliary beat in human airways and has cytotoxic effects on respiratory epithelial cells [3, 4]. Furthermore, PA-IL binds efficiently to Gb3-expressing cell lines [1], induces membrane invaginations, and selectively binds cardiac non-myocytes, but not atrial or ventricular cardiomyocytes [5]. The PA-IL-CAR chimeric molecule has recently demonstrated target-specific cytotoxicity against carcinoma cells overexpressing Gb3 (e.g., Burkit´s lymphoma-derived cell lines) [6].

References:

1. Blanchard *et al*, *J Mol Biol*, 2008, doi: 10.1016/j.jmb.2008.08.028
2. Bojar *et al*, *ACS Chem Biol*, 2022, doi: 10.1021/acschembio.1c00689
3. Bajolat-Laudinat *et al*, *Infect Immun*, 1994, doi: 10.1128/iai.62.10.4481-4487.1994
4. Mewe *et al*, *J Laryngol Otol*, 2005, doi: 10.1258/0022215054516313
5. Darkow *et al*, *Front Physiol*, 2020, doi: 10.3389/fphys.2020.00457
6. Meléndez *et al*, *Cell Mol Life Sci*, 2022, doi: 10.1007/s00018-022-04524-7

Guidelines for reconstitution of the lyophilized product

Wear protective gloves and clothing when handling the product. Respiratory protection should be worn when working with lyophilized lectin.

1. Allow the product to equilibrate to room temperature before opening the vial.
2. The product is offered in different amounts. For 1 mg, we recommend briefly centrifuging the vial and dissolving the whole lyophilisate. For 5 mg and 10 mg products, the desired quantity of freeze-dried protein can be transferred to a clean tube and dissolved there.
3. Add the desired solvent volume (see below for buffer recommendation), and allow the sample to reconstitute in the fridge (2 hours are recommended). If the undissolved particles are observed, let the sample dissolve at room temperature with gentle agitation, or for a prolonged period (overnight) in the fridge. Do not vortex.
4. Centrifuge the sample on a bench-top centrifuge (15 min, max speed) to remove the eventual insoluble material and check the concentration of the reconstituted lectin by measuring the absorbance at 280 nm (e.g., by Nanodrop).

In some instances, it may be challenging to dissolve the lyophilized powders completely, and the reconstitution efficiency may vary for different buffers. Therefore, 4GLYCO provides their customers with the output of testing their lectins in the most common solvents. The data can help customers select the appropriate buffer.

solvent	PA-IL dissolved
water (MilliQ)	95 %
20 mM Na acetate, pH 4.5	94 %
20 mM MES, pH 6.0	97 %
20 mM Tris, pH 7.5	97 %
50 mM Na borate, pH 8.5	96 %
20 mM Na acetate, 150 mM NaCl, pH 4.5	96 %
20 mM MES, 150 mM NaCl, pH 6.0	96 %
20 mM Tris, 150 mM NaCl, pH 7.5	95 %
20 mM Hepes, 150 mM NaCl, pH 7.5	95 %
PBS	98 %

The data were obtained by dissolving 0.5 mg of fresh, freeze-dried lectin in 500 µl of the buffers (2 hours, 4 °C), centrifuging, and measuring the lectin concentration in the supernatant spectrophotometrically.

Please note that the data are presented to compare different solvents. The absolute numbers may vary due to various factors.