Product specification

Product number:	**GL-007**
Product name:	**PA-IIL (LecB) lectin produced recombinantly in *E. coli*, unconjugated**
Form:	Lyophilized powder
Unit size:	1 mg, 5 mg, 10 mg
Sugar specificity:	L-fucose (see the detailed information below)
Protein sequence:	ATQGVFTLPANTRFGVTAFANSSGTQTVNVLVNNETAATFSGQSTNNAVIGTQVLNSGSSGKVQ VQVSVNGRPSDLVSAQVILTNELNFALVGSEDGTDNDYNDAVVVINWPLG
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-IIL (LecB, PA-II)	Molecular weight (monomer):	11731.8 Da
Organism:	*Pseudomonas aeruginosa*	Extinction coefficient:	6990 M^{-1} cm^{-1}
Expression host:	*Escherichia coli*	Oligomeric state:	tetramer
Tags:	no		

Carbohydrate specificity:

PA-IIL (LecB, formerly PA-II) lectin strongly binds L-fucose, its derivatives, and many fucosylated oligosaccharides, including Lewis and blood group ABO antigens. It has the highest preference for Lewis a, sialyl-Lewis a, and blood group H (type 2) epitopes; other Lewis/ABO oligosaccharides are less efficient ligands. PA-IIL does not recognize blood group A and B epitopes (type 1). Besides L-fucose, PA-IIL binds D-mannose and mannosylated glycans with weak affinity [1-2].

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

Stability:

Highly thermostable lectin. Stable in a variety of buffers with pH 4.0-10.0. 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 = 94 °C (DSC, 20 mM Tris, 150 mM NaCl, 0.1 mM $CaCl_2$, pH 7.5)

Applications and biological effects:

PA-IIL 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-IIL inhibits ciliary beat in human airways [3,4]. It induces BCR- and CD19-dependent activation of B cells [5], impairs keratinocyte fitness by abrogating growth factor signaling [6], causes integrin internalization, and inhibits epithelial wound healing [7]. Also, PA-IIL lectin stimulates human peripheral lymphocytes and murine splenocytes [8] and induces differentiation of the acute myeloid leukemia cell line THP-1 [9].

References:

1. Mitchell *et al*, *Proteins*, 2005, doi: 10.1002/prot.20330
2. Topin *et al*, *PLoS One*, 2013, doi: 10.1371/journal.pone.0071149
3. Adam *et al*, *Am J Respir Crit Care Med*, 1997, doi: 10.1164/ajrccm.155.6.9196121
4. Mewe *et al*, *J Laryngol Otol*, 2005, doi: 10.1258/0022215054516313
5. Wilhelm *et al*, *Sci Signal*, 2019, doi: 10.1126/scisignal.aao7194
6. Landi *et al*, *Life Sci Alliance*, 2019, doi: 10.26508/lsa.201900422
7. Thuenauer *et al*, *mBio*, 2020, doi: 10.1128/mBio.03260-19
8. Avichezer *et al*, *FEBS Lett*, 1987, doi: 10.1016/0014-5793(87)80757-3
9. Kühn *et al*, *Cell Death Discov*, 2015, doi: 10.1038/cddiscovery.2015.31

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-IIL dissolved
water (MilliQ)	100 %
20 mM Na acetate, pH 4.5	96 %
20 mM MES, pH 6.0	96 %
20 mM Tris, pH 7.5	100 %
50 mM Na borate, pH 8.5	97 %
20 mM Na acetate, 150 mM NaCl, pH 4.5	95 %
20 mM MES, 150 mM NaCl, pH 6.0	100 %
20 mM Tris, 150 mM NaCl, pH 7.5	98 %
20 mM Hepes, 150 mM NaCl, pH 7.5	100 %
PBS	100 %

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.