Product specification

Product number:	**GL-001**
Product name:	**AFL (FleA) 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:	STPGAQQVLFRTGIAAVNSTNHLRVYFQDVYGSIRESLYEGSWANGTEKNVIGNAKLGSPVAAT SKELKHIRVYTLTEGNTLQEFAYDSGTGWYNGGLGGAKFQVAPYSCIAAVFLAGTDALQLRIYAQ KPDNTIQEYMWNGDGWKEGTNLGGALPGTGIGATSFRYTDYNGPSIRIWFQTDDLKLVQRAYD PHKGWYPDLVTIFDRAPPRTAIAATSFGAGNSSIYMRIYFVNSDNTIWQVCWDHGKGYHDKGTIT PVIQGSEVAIISWGSFANNGPDLRLYFQNGTYISAVSEWVWNRAHGSQLGRSALPPA
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:	AFL (FleA)	Molecular weight (monomer):	34450.4 Da
Organism:	*Aspergillus fumigatus*	Extinction coefficient:	87320 M^{-1} cm^{-1}
Expression host:	*Escherichia coli*	Oligomeric state:	dimer
Tags:	no		

Carbohydrate specificity:

AFL (FleA) binds L-fucose and many fucosylated oligosaccharides with terminal fucose bound through α1-2, α1-3, and α1-4 linkages. AFL also binds core α1-6-fucosylated glycans, provided that fucose is sterically accessible. β-linked Fuc is recognized as well. In general, shorter and non-branched glycans are preferred over larger structures. AFL binds Lewis (Le^a, Le^b, Le^X, Le^Y) and blood group (A, B, and H) determinants. Lewis Y is a slightly preferred epitope [1-2].

Ion dependency: no Glycan array data available at functionalglycomics.org

Stability:

Stable in a range of neutral and slightly acidic buffers. Avoid extreme pH (below 4 or above 10) [3]. After reconstitution in neutral pH buffers, the protein should be stable for weeks in the fridge. Adding sodium azide (0.02%) is recommended to avoid microbial growth.

T_m = 51 °C (nanoDSF, PBS, pH 7.5)

Applications and biological effects:

AFL and closely related lectin AOL from *Aspergillus oryzae* are used in lectin blotting, fluorescence microscopy, flow cytometry, and lectin histochemistry experiments to detect the fucosylation of proteins, cells, and tissues. Also, they can be used to isolate fucosylated glycans (e.g., by lectin affinity chromatography). Both lectins are found in commercial lectin microarrays.

In addition, AFL was shown to have a pro-inflammatory effect on BEAS-2B [1], HCEC [4], A549, and MH-S [5] cells; AOL is known to induce anaphylactoid edema and mast cell activation through its interaction with fucose of mast cell-bound non-specific IgE [6].

References:

1. Houser *et al*, *PLoS One*, 2013, doi: 10.1371/journal.pone.0083077
2. Houser *et al*, *Acta Crystallogr D Biol Crystallogr*, 2015, doi: 10.1107/S1399004714026595
3. Houser *et al*, *Eur Biophys J*, 2021 doi: 10.1007/s00249-021-01497-6
4. Ballal *et al*, *Mol Cell Biochem*, 2017, doi: 10.1007/s11010-017-3050-95
5. Sakai *et al*, *Med Mycol*, 2019, doi: 10.1093/mmy/myx163
6. Yamaki *et al*, *Scand J Immunol*, 2011, doi: 10.1111/j.1365-3083.2011.02598.x

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	AFL dissolved
water (MilliQ)	96 %
20 mM Na acetate, pH 4.5	96 %
20 mM MES, pH 6.0	94 %
20 mM Tris, pH 7.5	87 %
50 mM Na borate, pH 8.5	100 %
20 mM Na acetate, 150 mM NaCl, pH 4.5	82 %
20 mM MES, 150 mM NaCl, pH 6.0	80 %
20 mM Tris, 150 mM NaCl, pH 7.5	94 %
20 mM Hepes, 150 mM NaCl, pH 7.5	97 %
PBS	95 %

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.