

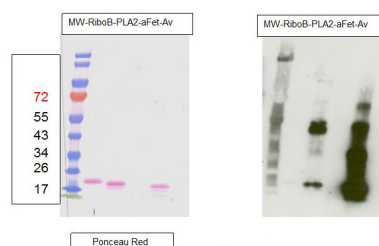
Product no **AS07 268****Anti-Fucose****Product information**

<b>Immunogen</b>	Core fucose residues bound to the N-glycan in alpha 1,3
<b>Host</b>	Rabbit
<b>Clonality</b>	Polyclonal
<b>Purity</b>	Immunogen affinity purified serum in PBS pH 7.4.
<b>Format</b>	Lyophilized
<b>Quantity</b>	50 µg
<b>Reconstitution</b>	For reconstitution add 50 µl of sterile water
<b>Storage</b>	Store lyophilized/reconstituted at -20°C; once reconstituted make aliquots to avoid repeated freeze-thaw cycles. Please remember to spin the tubes briefly prior to opening them to avoid any losses that might occur from material adhering to the cap or sides of the tube.
<b>Additional information</b>	Alpha (1,3) fucose is present not only in plants but also in some invertebrates (such as nematodes, bees, etc.) , However, cross-reaction with glycoproteins from these organisms is weaker than the one observed in plants, This sugar residue does not exist in mammals, in their endogenous glycoproteins

**Application information**

<b>Recommended dilution</b>	0.5 µg/ml (ELISA), 1 : 40 (IL), 1 µg/10 ml (WB)
<b>Expected   apparent MW</b>	10 - 100 for various glycoproteins
<b>Confirmed reactivity</b>	Higher plants, <i>Pheodactylum tricornutum</i> (diatom)
<b>Predicted reactivity</b>	Higher plants, <i>Phaeodactylum tricornutum</i>
<b>Not reactive in</b>	No confirmed exceptions from predicted reactivity are currently known
<b>Additional information</b>	<p>Controls:</p> <p>PLA2 (phospholipase 2 from bee venom) which contains only 1.3 fucose, Sigma, product number P9279.</p> <p>Type II - horseradish peroxidase which contains 1.2 Xylose and 1.3 fucose, Sigma, product number P8250.</p> <p>The antibody does not recognize alpha 1,6-fucose.</p>
<b>Selected references</b>	<p><a href="#">Xu et al. (2023)</a>. The Golgi-localized transporter OsPML4 contributes to manganese homeostasis in rice. <i>Plant Sci.</i> 2023 Dec 2:339:111935.doi: 10.1016/j.plantsci.2023.111935.</p> <p><a href="#">Zhang et al. (2018)</a>. An important role of L -fucose biosynthesis and protein fucosylation genes in Arabidopsis immunity. <i>New Phytol.</i> 2018 Dec 15. doi: 10.1111/nph.15639.</p> <p><a href="#">Jansing et al. (2018)</a>. CRISPR/Cas9-mediated knockout of six glycosyltransferase genes in <i>Nicotiana benthamiana</i> for the production of recombinant proteins lacking -1,2-xylose and core -1,3-fucose. <i>Plant Biotechnol J.</i> 2018 Jul 3. doi: 10.1111/pbi.12981.</p> <p><a href="#">Nakanishi et al. (2017)</a>. Protection of Human Colon Cells from Shiga Toxin by Plant-based Recombinant Secretory IgA. <i>Sci Rep.</i> 2017 Apr 3;7:45843. doi: 10.1038/srep45843. (ELISA)</p> <p><a href="#">Hanania et al. (2017)</a>. Establishment of a tobacco BY2 cell line devoid of plant specific xylose and fucose as a platform for the production of biotherapeutic proteins. <i>Plant Biotechnol J.</i> 2017 Feb 3. doi: 10.1111/pbi.12702.</p> <p><a href="#">Ebert et al. (2015)</a>. Identification and Characterization of a Golgi-Localized UDP-Xylose Transporter Family from Arabidopsis. <i>Plant Cell.</i> 2015 Mar 24. pii: tpc.114.133827.</p> <p><a href="#">Lehtimäki et al. (2014)</a>. Posttranslational modifications of FERREDOXIN-NADP+ OXIDOREDUCTASE in Arabidopsis chloroplasts. <i>Plant Physiol.</i> 2014 Dec;166(4):1764-76. doi: 10.1104/pp.114.249094</p> <p><a href="#">Balet et al. (2010)</a>. N-glycans of <i>Phaeodactylum tricornutum</i> diatom and functional characterization of its N-acetylglucosaminyltransferase I enzyme. <i>J Biol. Chem.</i> Dec 17.</p>

## Western blot

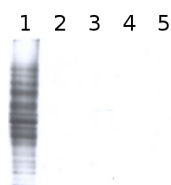


The Anti-fucose antibodies exhibit a positive response with PLA2 and Avidin and a negative one with asialofetuin. This suggests this antibody recognizes only 1,3 fucose epitopes.

For each sample, 1 µg of purified protein was denatured with leammli sample buffer at 100°C for 10 minutes loaded on a Bis-Tris gel 4-12% and blotted 1h15 to nitrocellulose membrane using semi-dry transfer. Blots were saturated in TBST over night with agitation. Blot was incubated for 2 hours with primary antibody at a dilution of 1:5000 in TBST at RT with agitation. The antibody solution was discarded and after quick washes with TBST, the blot was washed 3X15 min in TBST. Blot was incubated in secondary antibody (Goat anti Rabbit IgG, HRP conjugated [AS09 602](#) lot 1707 from AGRISERA) diluted to 1:50 000 in TBST for 1 hour at RT with agitation. The blot was washed as above, plus 1 wash in TBS for 10 min at RT with agitation. The blot was developed for 5 min with chemiluminescent detection reagent. Exposure time was 5 seconds.

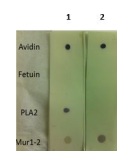
**RiboB:** RiboNuclease B contains only oligomannosidic N-Glycans. Used here as a negative control. **PLA2:** Phospholipase A2 from honey bee venom (*Apis mellifera*) contains 1,3 Fucose residues. **aFet:** Asialofetuin is a glycoprotein with 1,6 fucose. Used here as a negative control. **Av:** recombinant Avidin expressed in corn contains 1,3 Fucose residues.

Courtesy Dr. Muriel Bardor, University of Rouen, France



Total cell extract from *Arabidopsis thaliana* wild type (1) and cell extracts from different mutants defective in fucosyltransferases (2-5) (data not published yet).

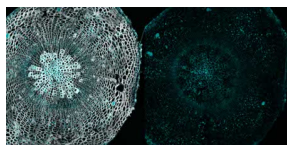
Primary antibody has been used at 10 µg/10 ml of incubation buffer. Detection has been done using enhanced chemiluminescence (ECL).



Dot blot reaction of anti-Fucose and anti-Xylose antibodies with various controls: Avidin (Fuc+/Xyl+), Fetuin (Fuc-/Xyl-), PLA2 (Fuc+/Xyl-) and Mur1-2 (Fuc-/Xyl+). 2 µl of each extract were spotted on a nitrocellulose membrane, placed on top of 2 WHATMAN filters (one soaked in TBS-T) and dried for 1.5 h at RT. The membrane was blocked for 30 min with 2% low-fat milk powder in TBS-T (0.1% TWEEN 20) and incubated with anti-Fucose(1) ([AS07 268](#), 1:1000) or anti-Xylose(2) ([AS07 267](#), 1:1000) for 30 min and then with secondary anti-rabbit(1:1000) antibody (ALP conjugated, recommended secondary antibody [AS09 607](#)). Membrane was washed with TBS-T 3 x 10 minutes before reaction development using alkaline phosphatase reagent BCIP®/NTB premixed solution (Sigma, Prod. No. B6404).

Please follow this link for a more detailed [Dot-Blot](#) protocol

## Immunolocalization



Immunolocalization using anti-fucose antibodies on a 40µm vibratome section of a 21 day old *Arabidopsis thaliana* hypocotyl (wood). Fixation: 4% formaldehyde fixation in 1X PME (100 mM PIPES, 1 mM MgSO<sub>4</sub>, 2 mM EGTA); blocking: 5 % BSA; primary antibody dilution: 1:40; secondary antibody: 1:300 AlexaFluor568 from Invitrogen (Life Technologies), Alexa Fluor® 568 Goat Anti-Rabbit IgG (H+L); Left panel, immunolabel channel (cyan) overlaid on grayscale channel (0.001% Calcofluor white which stains cellulose and hemicellulose). Right panel, only immunolabelling

Courtesy of Dr. Hardy Hall and Dr. Urs Fischer, Umeå Plant Science Centre, Sweden