



## Description

The  $\alpha 2,3\_ \alpha 2,6$  LEctPROFILE® kit is designed to study the sialylation and more precisely the ratio  $\alpha 2,3/\alpha 2,6$  of sialic acid motifs. Indeed, the kit is composed of two specific lectins: (1) the *Sambucus Nigra* Agglutinin (SNA) that binds preferentially to sialic acid  $\alpha$ -2,6 Gal (found in N-glycans) or sialic acid  $\alpha$ -2,6 GalNAc (found in O-glycans) but not on sialic acid  $\alpha$ -2,3 Gal oligosaccharides, and (2) the *Maackia amurensis* leucoagglutinin (MAA) is inhibited by low concentration of 2,3-sialyllactose (NeuAc2,3Gal $\beta$ 1,4Glc), but not inhibited by either 2,6-sialyllactose or free NeuAc.

## Applications

➡  **$\alpha 2,3/\alpha 2,6$  ratio determination of glycoproteins**

Aguedo, J. *et al.*<sup>1</sup>

The ratio  $\alpha 2,3/\alpha 2,6$  sialic acid motifs was determined through reference glycoproteins (fetuin and transferrin) known to have specific sialic acid structures and compared to the MALDI-TOF/MS results. The interactions of these glycoproteins with SNA and MAA lectins were performed on the native form, or after neuraminidase treatments (See *graphes next page*).

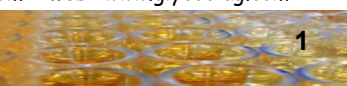
Name	Glycosylation profil according to literature
Fetuin from foetal calf serum (BioRad, Ref :4430-2204)	- 3N- an 3 O-linked (mucine-type)glycans. - Complex glycans with NeuAc ( $\alpha 2,6$ & $\alpha 2,3$ )
Human Transferrin (Sigma Aldrich, Ref T3309)	- 2 N-linked complex glycans containing NeuAc ( $\alpha 2,6$ & $\alpha 2,3$ )

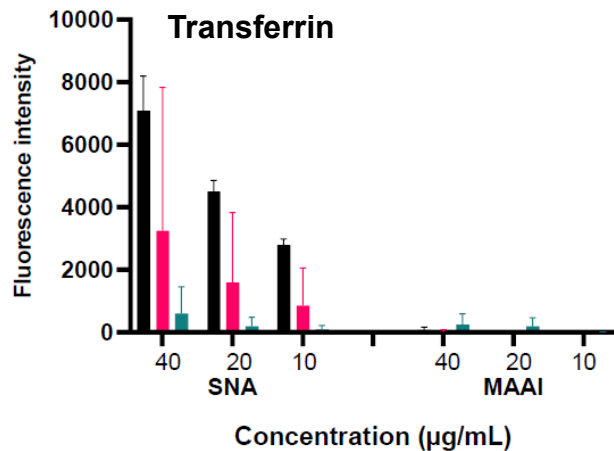
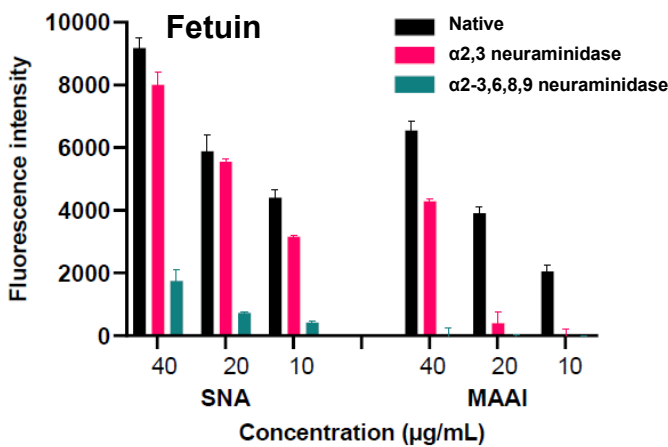
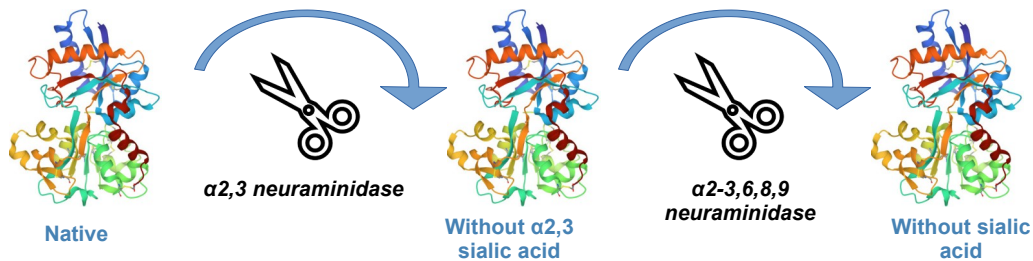
## Results

**Fetuin:** - The  $\alpha 2,3/\alpha 2,6$  sialic acid ratio obtained is 46:54. This ratio is in total accordance with the bibliography that report value of  $\alpha 2,3/\alpha 2,6$  on fetuin from 38:62 to 49:51.

**Transferrin:** - None of  $\alpha 2,3$  structures were detected due to the absence of interaction with MAA. However, transferrin is known to have  $\alpha 2,3$  motifs. Our hypothesis for the absence of interaction with MAA, is due to a lack of accessibility of the  $\alpha 2,3$  glycan structures on native glycoprotein. Indeed, after the treatment of transferrin in denaturing conditions, we observed on the denatured transferrin interactions with MAA that confirm the presence of  $\alpha 2,3$  glycans.

The use of  $\alpha 2,3\_ \alpha 2,6$  LEctPROFILE® kit is a complementary method to the structural analysis that enable to clearly identify the glycan motifs accessible on glycoproteins for biological interactions.

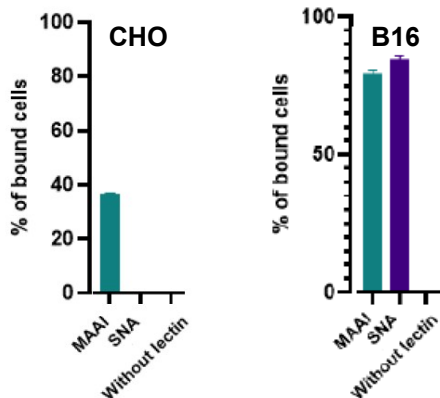




### Cells glycosylation study

Vena, F. *et al.*<sup>2</sup>

The  $\alpha 2,3_{\alpha 2,6}$  LEctPROFILE® kit is an easy tool to follow the glycosylation profil of cells due to it's high specificity of recognition for  $\alpha 2,3$  and  $\alpha 2,6$  sialic acid motifs. For example CHO cells are known to express only  $\alpha 2,3$  glycans. In other part, melanoma B16 is a murine tumour cell line used for research as a model for human skin cancers. Both sialic acid residues are expressed on these cells. The interactions obtained with  $\alpha 2,3_{\alpha 2,6}$  LEctPROFILE® kit with CHO and melanoma B16 are in total accordance with the sialylation data. As it is know that the sialylation profiles are evolving during the cancer, the  $\alpha 2,3_{\alpha 2,6}$  LEctPROFILE® kit constitutes a powerful way to follow the progression of the disease.





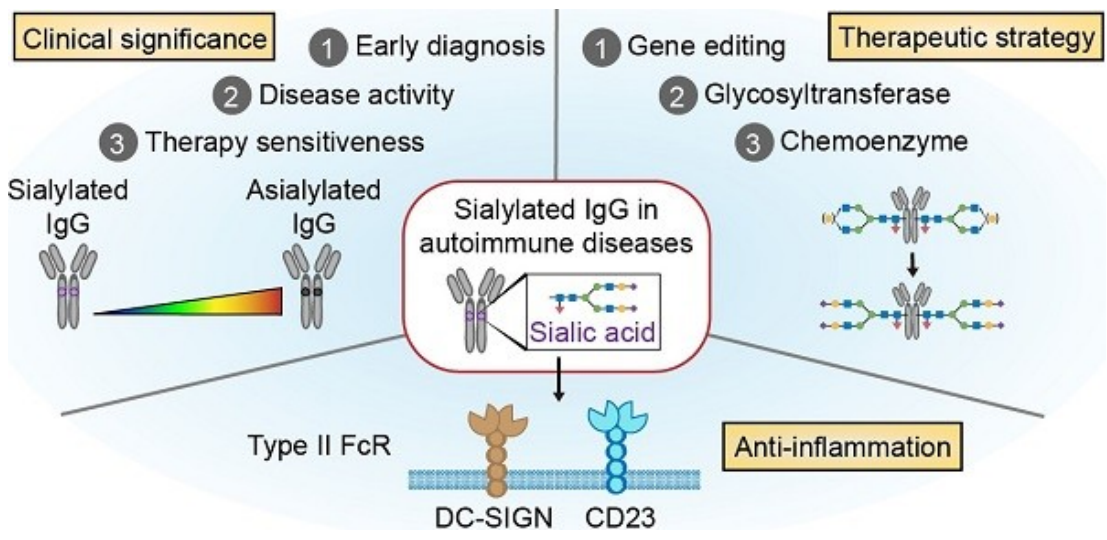
➔ Follow the sialylation level of IgG contained in biological fluid

Li, D. *et al.*<sup>3</sup>

Recently, it was proved that the IgG sialylation level of a wide variety of autoimmune diseases changes during the development and progression of the disease. Indeed, low levels of sialylated IgG glycans in serum have been reported as glycomarkers in a number of autoimmune diseases.

The  $\alpha 2,3_{\alpha 2,6}$  LEctPROFILE® kit can be used to :

- facilitate diagnosis by measurement of sialylation level of IgG contained in serum of patients ;
- monitor disease progression ;
- evaluate therapeutic efficacy of a strategy used to recover the sialylation level.



**References**

1. J. Aguedo, F. Vena, L. Landemarre, J. Tkac, Rapid and high-throughput methods for discrimination of sialic acid linkages in glycoproteins, Group Français des Glycosciences, **2022**, France.
2. F. Vena, LEctPROFILE kits: towards quality control and new potential applications, GLYcoDiag, thesis defence, 13<sup>th</sup> Décembre 2022, Orléans.
3. D. Li, Y. Lou, Y. Zhang, S. Liu, J. Li. *Sialylated immunoglobulin G: a promising diagnostic and therapeutic strategy for autoimmune diseases*, *Theranostics*, **2021**, 11, 5430-5446.

