GlycoImage®

GALAB Technologies

GlycoImage® Lectin Array Kit

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User Guide

Edition 07/09

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GlycoImage® Lectin Array Kit Contents

Biotinylation reagents

Biotin-NHS	1 vial
DMSO	1 vial
Labeling Buffer	1 vial
Dialysis Buffer	1 bottle (25mL)

Assay reagents

Enzyme conjugate	1 vial (30 µL)
Substrate	2 vials (à 42 mg)
Glycoprotein biotin (control)	1 vial (2 mL)
Assay Buffer	2 bottles (à 25mL)
Equilibration buffer	1 bottle (25mL)
Detection Buffer	1 bottle (30mL)
Lectin Array Plate	1 x 384well

Storage

Store the kit at 2°C to 8°C. Solutions contain 0.05% sodium azide as a preservative. The stability of the kit is at least 6 month.

! For best results please maintain a constant temperature of 25°C (+/- 2°C) during incubation cycles and measurement. Avoid a direct exposure of the plate and the reagents to sun light.
Buffer Solutions have to be kept at 2°C to 8°C during assay procedure.

Safety- and Product information

Handling of chemicals always requires wearing of safety clothes. For more information please read the material safety data sheets (MSDS). MSDS are provided on CD or at www.galab.de/technologies

The kit and the kit components are not for diagnostic, pharmaceutical or in-vivo use.

Instrumental and material requirements

- microplate reader for measurements at 405nm
- pipettes/ multichannelpipettes (8-/12-channel)/ multistepper (volume range: 10 µL- 50 µL)
- dialysis membrane (MWCO depends on your sample)

Biotinylation of samples

1 Biotin-NHS is provided as a dry substance.
The content of one vial (4mg) is sufficient for the biotinylation of 2mg protein or sample.

Dissolve the content of the vial by adding 200 µl DMSO.
Dissolved reagent is not stable and should not be stored.

2 Dilute your Sample with PBS to a concentration of 1,1 mg/mL..

3 Add 1 part of biotin to 9 parts of your sample.

For example:
Add 10µL of the biotin solution to 90 µL of sample solution.

4 Incubate the reaction mixture for one hour.

5 Dialyse the reaction mixture against the 1x concentrated Dialysis Buffer.
This will block unreacted reagent and separate free NHS-groups.

Sample storage

They can be stored at -20°C for long-term.

Sample dilution for lectin array applications

After biotinylation, the samples are 1000-fold concentrated (ß= 1mg/mL)

(Dilute 10µL of the conjugate with 9.990 µL Assay Buffer up to 10 mL (ß=1µg/mL))

Preparation of lectin array reagents

The kit offers the possibility for measurement of 4 samples per microplate (in duplicates).
The estimated time for running the protocol is 4-5 h. Preparations are calculated for one plate.

1. The Assay Buffer is a 20-fold concentrated solution.
Dilute the content of one bottle completely with 475 mL deionised water to a volume of 500mL.
The second bottle can be used, when working with an automated microplate washer.
Please keep the Assay Buffer at 2°C to 8°C even during the assay procedure.

2. The Equilibration Buffer is 2-fold concentrated.
Dilute the content of one bottle completely with 25 mL deionised water to a volume of 50 mL.

3. The Detection Buffer is „ready-to-use".

4. Glycoprotein Conjugate
The conjugate is 1000-fold concentrated.
(Dilute 10µL of the conjugate with 9.990 µL Assay Buffer Solution up to 10mL)

5. Enzyme Conjugate
The enzyme conjugate is 1000-fold concentrated.
(Dilute 10µL of with 9990 µL Assay Buffer)

6. Substrate
Dissolve the whole content (42 mg) of one substrate vial in 15mL Detection Buffer (ß=2,8mg/mL).
Prepare fresh solution immediately before measurement.

Plate Layout

No.		Lectin	Specificity
1 + 13	BSA	bovine serum albumine	used as a blank control
2+14	ConA	Concanavalin A	mannose (N-glycan core)
3+15	LCH	lens culinaris hemagglutinin	
4+16	GNA	galanthus nivalis agglutinin	
5+17	PNA	peanut agglutinin	galactose
6+18	ECL	erythrina cristagalli lectin	
7+19	PHA-E	phaseolus vulgaris	N-Acetylglucosamine
8+20	WGA	wheat germ agglutinin	
9+21	SNA	sambucus nigra agglutinin	N-Acetylneuraminic acid
10+22	MAL	maackia amurensis lectin	
11+23	AAL	aleuria aurantia lectin	fucose
12+24	UEA	ulex europaeus agglutinin	

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	Sample 1 (in duplicate)												Sample 5 (in duplicate)											
B																								
C	Sample 2 (in duplicate)												Sample 6 (in duplicate)											
D																								
E	Sample 3 (in duplicate)												Sample 7 (in duplicate)											
F																								
G	Sample 4 (in duplicate)												Sample 8 (in duplicate)											
H																								

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:
up to P

Assay Protocol

1

Add 20µL/ well of your sample.

Incubate the plate at 25°C for 2 hours.
Avoid a direct light exposure.

2

Use cold Assay Buffer to wash your plate.
First, empty your plate. Add now 100 µL /well of the Assay Buffer to the plate and empty the plate again.
Repeat it 5 times. After the last cycle, drop the plate out on a tissue.

3

Add 50µL/ well of the enzyme conjugate.
Incubate the plate at 25°C for 1 hour. Avoid a direct light exposure.

4

Use cold Assay Buffer to wash your plate.
First, empty your plate. Add now 100 µL /well of the Assay Buffer to the plate and empty the plate again.
Repeat it 3 times with Assay Buffer and 2 times with Equilibration Buffer. After the last cycle, drop the plate out on a tissue.

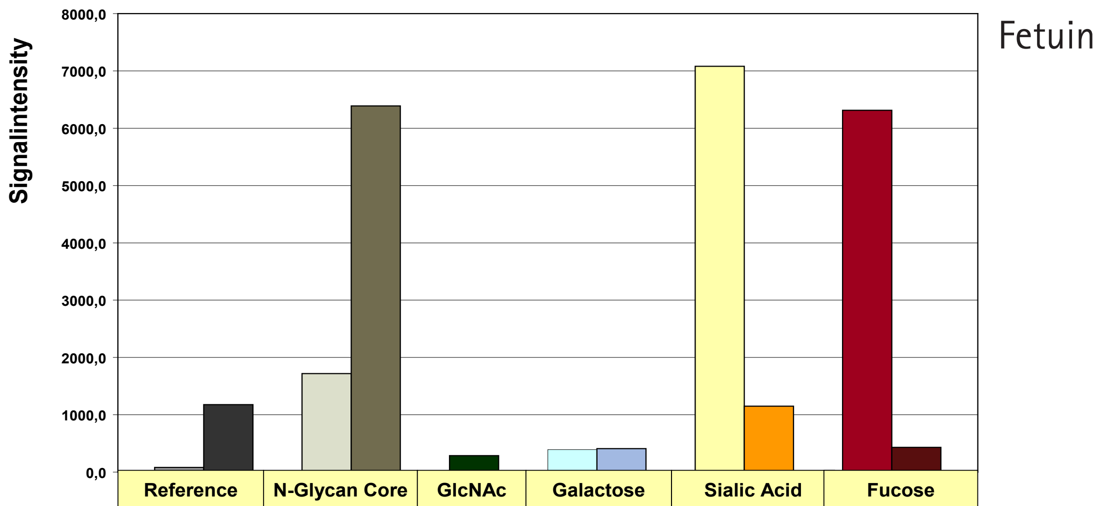
5

Prepare your microplate reader
(kinetic measurement, run time: 30 Min, intervals: 5 Min)

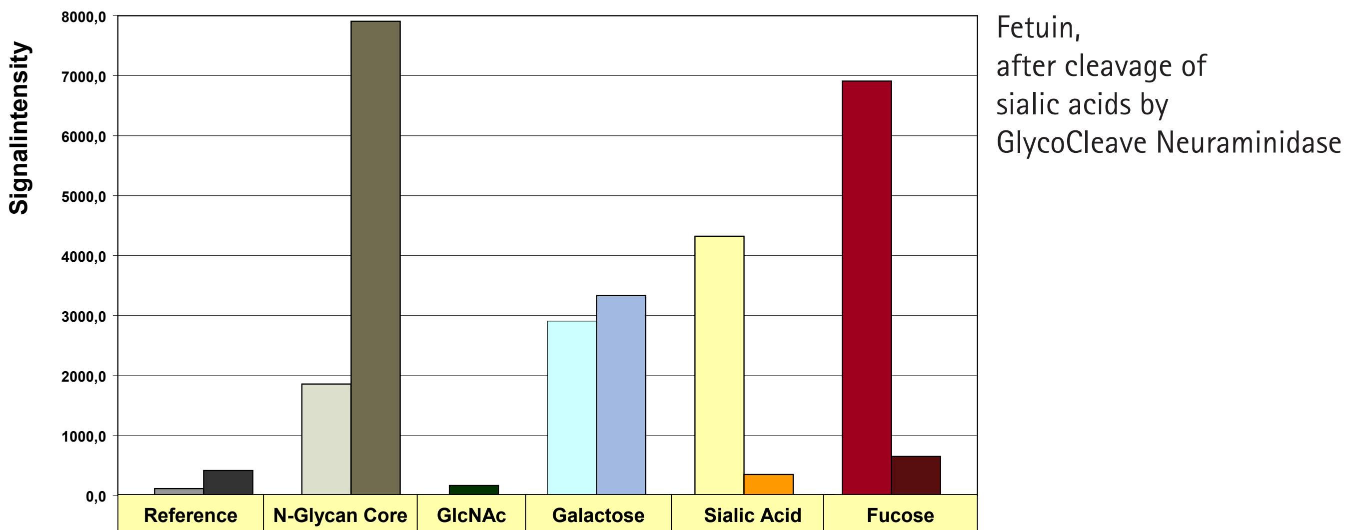
6

Add 20µL/ well of the substrate solution.
Immediately after adding the solution start your measurement

Application: Determination of the degree of desialylation after treatment with GlycoCleave® Neuraminidase



Fetuin



Fetuin,
after cleavage of
sialic acids by
GlycoCleave Neuraminidase

The results show a fingerprint of the determined samples. The samples presented in the charts above are glycoproteins. Chart 1 shows the fingerprint of bovine fetuin, a sialylated protein. Chart 2 presents the fingerprint of bovine fetuin after neuraminidase treatment. The fingerprint (chart 2) shows an increase of signal intensity on galactose-binding lectins on the one hand, on the other hand there is an decrease in signal intensity on sialic-acid specific lectins.

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