rekom biotech

High Quality Raw Materials
for the IVD Manufacturing Industry

Immunoassay blocker for anti-cross-reactive carbohydrate determinants (CCD) antibodies

Cross-reactive carbohydrate determinants (CCD) are glycan structures attached to proteins during post-translational modifications. In addition to the CCD structures of glycosylated proteins from plants or invertebrates, which are immunogenic (as they differ from those of human glycoproteins), there are also glycoproteins from several yeast species which have adapted to colonize human tissue. One example is the endo-saprophytic yeast *Candida albicans*, which can invade human tissues in immunosuppressed patients, leading to frequent nosocomial systemic infections.

Among these virulence attributes critical for survival under changing environmental conditions, is the yeast cell wall containing large amounts of carbohydrates and carbohydrates covalently linked to a noncarbohydrate moiety classified as glycoconjugates, either glycoproteins or glycolipids. Despite similarities in the early steps of processing, the mature structure of glycans differs substantially between yeasts and mammals. Depending on the species, fungal high mannose glycans contain distinctive modifications, such as the addition of mannosyl phosphate and -linked mannose. These IgG antibodies against the -linked mannose are frequently present among healthy individuals without regard to age, race, or gender.

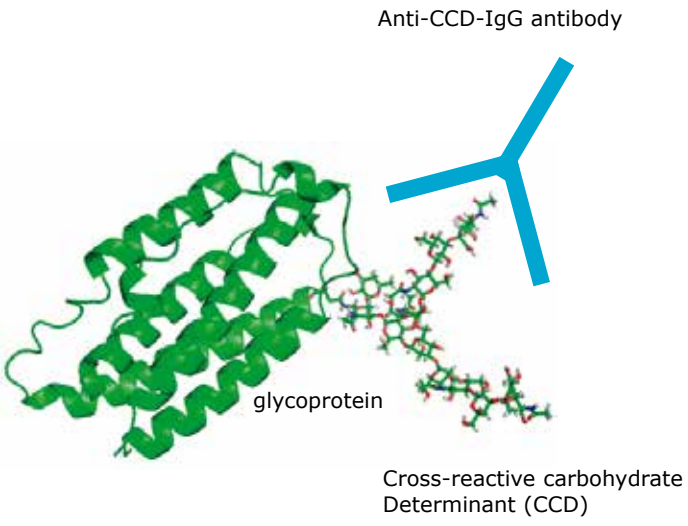
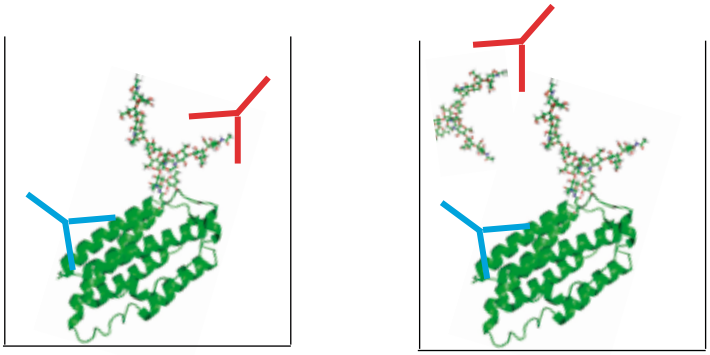


Figure 1. The glycoprotein contains a carbohydrate structure which can act as a cross-reactive carbohydrate determinant (CCD), interacting with IgG antibodies anti-mannan present in some normal human sera.

The β-1,2-linked Man residues have been established to be potent antigens for the adaptive immune response and to elicit specific infection protective antibodies. These IgG antibodies present in the normal human sera can cross-react to glycoproteins which contain these β-1,2-linked Man residues in their glycan structure as the glycoproteins produced in *Pichia pastoris* (Mille *et al.*, 2008).

The methylotrophic yeast *Pichia pastoris*, currently reclassified as *Komagataella pastoris*, has become a substantial workhorse for biotechnology, especially for heterologous protein production. One of the main benefits of this system compared with bacterial systems lies in the fact that glycosylated proteins can be produced, which help to a correct folding of the secreted protein. Successful expression of many industrial enzymes as well as pharmaceutically relevant proteins has rendered the methylotrophic yeast *P. pastoris* one of the most suitable and powerful protein production host systems, and without doubt, the currently most inexpensive eukaryotic expression system in market.

The blocker eliminates anti-CCD IgG antibodies from patient serum, which increases the specificity of the result. The use of the anti-CCD-blocker IgG only is indicated when antibodies against CCD structures are present in the patient serum.



Without blocker

A

Antibodies present in the serum react with different parts of the protein, both epitopes and the carbohydrate fraction containing β-1,2-mannose bonds

With blocker

B

The blocker kidnaps the Ab of the serum, which recognises the glycosidic part of the recombinant protein; therefore, only the Ab against epitopes remain in the serum, and the diagnosis specifies.

Figure 2. As some human normal sera contains IgG antibodies against mannan, they can interact with the CCD structures of the proteins produced in *Pichia* (A). With addition of the blocker, the anti-CCD antibodies will be kidnapped, and the specificity of the assay will increase.

ELISA ASSAY IN PRESENCE AND ABSENCE OF BLOCKER

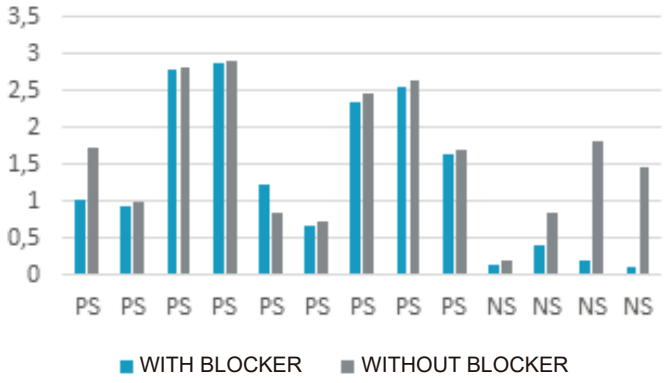


Figure 3. Indirect IgG ELISA assay was performed with SAG1 of *T. gondii* (RAG0030) produced in *P. pastoris*. The coating was carried out with 1 µg/ml of protein. The assays were performed with (blue) and without (grey) blocker incubation. PS means positive serum and NS means negative serum.

BLOCKER ADDITION

The procedure will be as follows:

Reconstitute with 0.692 ml of water, shake at RT for 5 min to obtain a homogeneous solution, considering that part of the cake can be on the container's glass walls. If the complete content is not immediately used, it can be aliquoted and stored at -20ºC.

Add the needed quantity to the sample diluent to reach the final blocker concentration between 5 and 80 µg/ml (0.5 to 8 µg/100 µl). A larger blocker quantity will be required when the serum dilution is minor. Incubate at RT for 10 min. Afterwards, the assay can be proceeded as usual.

Figure 4. Reconstitution and working procedure.

NAME	CAT NUMBER	DESCRIPTION
Blocker for anti-cross-reactive carbohydrate determinants (CCD) antibodies	**SOR0001**	Solution of several glycoconjugates

Pack size: 1 mg
Format: lyophilised

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