Anti-cross-reactive carbohydrate determinants (CCD) sorbent

CATALOG NUMBER: SOR0001 **LOT NUMBER:** #

1. **DESCRIPTION:** solution of several glycoconjugates.
2. **PRESENTATION:** dry powder
3. **RECONSTITUTION:** 0.94 ml of sterilised water. A final concentration of approx. 1 mg/ml will be obtained. The solubilisation of the cake should be developed for 5 min to allow a homogeneous protein solution, considering that part of the cake can be on the glass-walls of the container. The final color of the solution can be pale green.
4. **QUANTITY:** 1 mg (Bradford assay).
5. **ACTIVE QUANTITY:** 25 µl of sorbent (25 µg) have been used per 200 µl of diluted serum (dil 1:200) in ELISA assay. Quantities between 17 and 531 µg/ml in normal human sera have been described. Each end user should carry out his own titration for his particular application.
6. **STORAGE:** sorbent is shipped at room temperature. Upon arrival, it should be stored at 4ºC or -20ºC. Once reconstituted, it should be aliquoted to avoid repeated freezing and thawing cycles and stored at -20ºC to -80ºC.

BIBLIOGRAPHY:

Mille C, Bobrowicz P, Trinel PA, Li H, Maes E, Guerardel Y, Fradin C, Martinez-Esparza M, Davidson RC, Janbon G, et al. Identification of a new family of genes involved in β-1,2-mannosylation of glycans in Pichia pastoris and Candida albicans. J Biol Chem 2008. 283(15): 9724–9736.

Bradford, MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. Anal Biochem. 1976, 131:499-503.

Important Notes: During shipment, small volumes of product will occasionally become entrapped in the seal of the product vial. For products with volumes of 200 µl or less, we recommend gently tapping the vial on a hard surface or briefly centrifuging the vial in a tabletop centrifuge to dislodge any liquid in the containers cap.

Although *P. pastoris* is destroyed during purification, the product preparation should be handled as potentially infectious.

FOR RESEARCH AND COMMERCIAL USE *IN VITRO*: not for human *in vivo* or therapeutic use.

Rekom Biotech S.L. – BIC-Granada, Avda. Innovación, 1 – 18016, Granada (Spain) Tel: +34 958 63 70 85
E-mail: info@rekombiotech.com – Web: www.rekombiotech.com – An ISO 9001 Certified Company

BACKGROUND

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 β-linked mannose. These IgG antibodies against the β-linked mannose are frequently present among healthy individuals without regard to age, race, or gender.

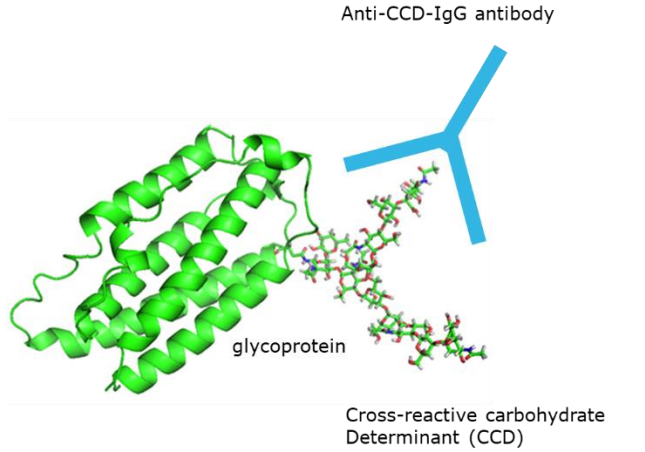
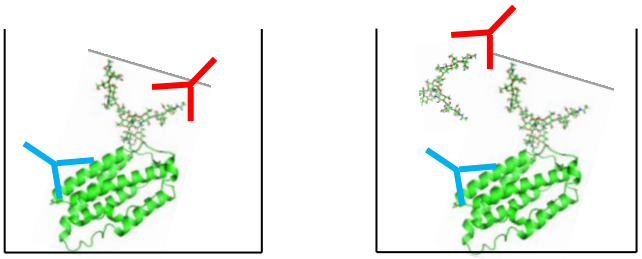


Figure 1. The glycoprotein contains a carbohydrate structure which can act as a cross-reactive carbohydrate determinant (CCD), interacting with IgG antibodies anti-mannan present in some normal human sera.

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



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

B

The sorbent 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 sorbent, the anti-CCD antibodies will be kidnapped, and the specificity of the assay will increase.

ELISA ASSAY IN PRESENCE AND ABSENCE OF SORBENT

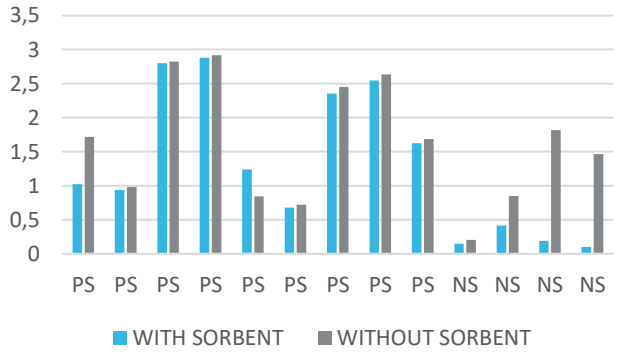


Figure 3. Indirect IgG ELISA assay was performed with SAG1 (RAG0030) produced in *P. pastoris*. The coating was carried out with 1 ug/ml of protein. The assays were performed with (blue) and without (grey) sorbent incubation. PS means positive serum and NS means negative serum.

Rekom Biotech S.L. – BIC-Granada, Avda. Innovación, 1 – 18016, Granada (Spain) Tel: +34 958 63 70 85
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SORBENT ADITION

The procedure will be as follows:

Reconstitute with 0.94 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 25 µl (25 µg) of the reconstituted sorbent to 200 µl of the diluted serum (1:200). Mix at RT for 10 min. Then, the assay can be proceeded as usual.

Figure 4. Reconstitution and working procedure.

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