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

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

1. **DESCRIPTION:** solution of several glycoconjugates.

2. **PRESENTATION:** dry powder (stabilized with 5% trehalose)

3. **RECONSTITUTION:** with 0.670 ml of sterile double-distilled water, a final concentration of 1.37 mg/ml will be obtained. The solubilisation of the cake should be developed for 15 min to allow a homogeneous solution, considering that part of the cake can be on the glass-walls of the container. Please keep in mind that the final volume of the reconstituted solution may differ from the reconstitution volume mentioned in the instructions due to the hygroscopic nature of trehalose. As the blocker is reconstituted, the final volume may slightly increase to reach the specified amount mentioned in the certificate of analysis. Upon reconstitution, leave the solution at least 15 min homogenizing with a mild agitation at 4ºC. Avoid vigorous shaking that can cause foaming. After those minutes, centrifuge the vial to ensure that all the product remains at the base and do not lose any of it on the walls. It is recommended that the users carry out their absorbance determinations to avoid concentration variabilities due to the equipment used, mainly in reproducibility analysis. The final colour of the solution can be pale green.

4. **QUANTITY:** 1 mg (Bradford assay).

5. **RECOMMENDED CONCENTRATION:** for best performance, SOR0001 should be included as part of the sample diluent, at a recommended final concentration range of 5 to 80 μg/ml, depending on the anti-CCD Ab quantity in a specific serum. Each end user should carry out the titration for their particular application.

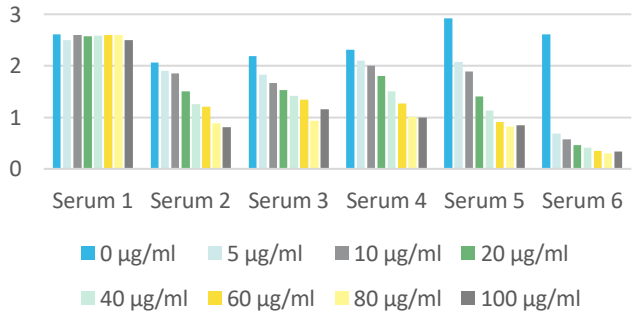

Figure 1. Blocker Performance. The graphic corresponds to an indirect IgG ELISA, using a protein produced in *Pichia pastoris*, coated at 1 μg/ml. The ELISA assay was developed by using general population sera. A final concentration blocker between 0 to 100 μg/ml was used. As can be seen, the blocker does not affect the performance of a serum with no anti-CCD Ab population (serum Nº 1), and some positive anti-CCD Ab serum requires more (serum Nº 4) or less (serum Nº 6) quantity of blocker to neutralize the anti-CCD Ab population completely.

6. **STORAGE:** blocker is shipped at room temperature. Upon arrival, it should be stored at 4° to -20°C in vertical position, avoiding all possible humidity and maintaining the vials dry. Once reconstituted, it should be aliquoted to avoid repeated freezing and thawing cycles and stored at -20ºC to -80ºC.

7. **APPLICATION:** Some normal human sera contain IgG antibodies against mannan from various pathogenic Candida species. This allows them to interact with CCD structures of the proteins produced in *Pichia pastoris*. With the addition of this blocker, the anti-CCD antibodies will be kidnapped, so the assay's specificity will increase (see Background below).

8. **BLOCKER ADDITION:** The procedure will be as follows:

Reconstitute with 0.670 ml of water, shake for 15 min at 4ºC 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 2. Reconstitution and working procedure.

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

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

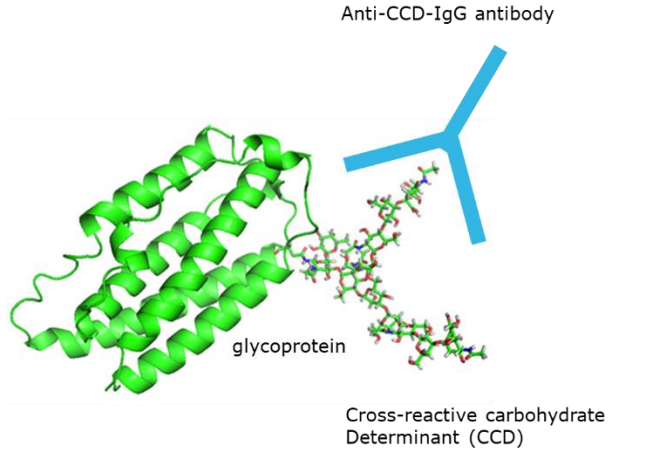
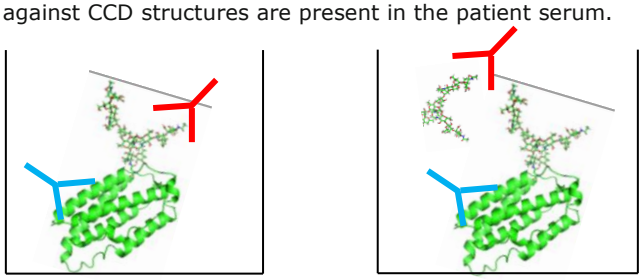


Figure 2. The glycoprotein contains a carbohydrate structure which can act as a cross-reactive carbohydrate determinant (CCD), interacting with IgG antibodies anti-mannan present in some normal human sera.

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 3. 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

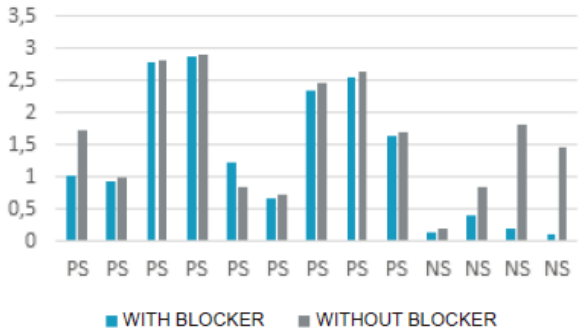


Figure 4. Indirect IgG ELISA assay was performed with SAG1 of *T. gondii* (RAG0030) produced in *P. pastoris*. The coating was carried out with 1 ug/ml of protein. The assays were performed with (blue) and without (grey) blocker incubation. PS means positive serum and NS means negative serum.

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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.

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