Recombinant hepatitis B virus core antigen assembled as capsid-like particles

CATALOG NUMBER: RAG0056 **LOT NUMBER:** #

RECOMBINANT ANTIGEN: recombinant hepatitis B virus core antigen (Rolland *et al.*, 2001).

DESCRIPTION: the recombinant HBV core antigen subtype ayw4 has been prepared as a recombinant capsid-like particles. It comprises the 149-residue assembly domain and a 34-residue arginine-rich domain.

PRESENTATION: liquid protein solution

SOURCE: *Escherichia coli*

SPECIFIC ANTIBODY (CALIBRATOR): Polyclonal antibody against HBcAg for Hepatitis B (HBV) (Rekom Biotech catalog reference PAB0008)

MOLECULAR WEIGHT: determined by SDS-PAGE, the protein band is between molecular markers of 25,000-35,000 Da, while relative molecular mass calculated from amino acid sequence is 25,399.85 Da. Under non-reducing conditions, forms high-molecular weight particles.

BATCH COMPOSITION:

COMPONENTS	COMPOSITION
his-HBcAg	recombinant antigen with a his-tag in its N-terminus
Storage buffer	20 mM phosphate buffer pH 8, 0.15 M NaCl, 0.26 M trehalose and 5 mM EDTA

QUALITY CONTROL:

1. PROTEIN CONCENTRATION DETERMINED ESPECTROPHOTOMETRICALLY

DO_{280} = 1.37
$A_{0.1\,\%}$ (=1 g/l) = 1.228
CONCENTRATION*: 1.11 mg/ml

* The measurement of the protein concentration has been performed with the theoretical extinction coefficient of the recombinant protein obtained from Gill and vonHippel, 1989. It is recommended that the users carry out their absorbance determinations to avoid equipment variabilities regarding final concentration, mainly in reproducibility analysis.

2. PURITY CONTROL IN SDS-PAGE: 15%

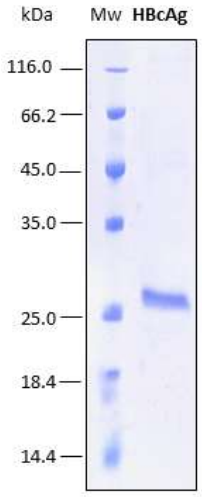

Figure 1. SDS-PAGE analysis (15%) of 4 µl of recombinant HBcAg. Purity is approx. 95% as determined by gel electrophoresis.

3. DISCRIMINATION OF PRE-VALIDATED SERA BY AN INDIRECT ELISA ASSAY

The cut-off has been suggested about an "in-house" ELISA kit performed in Rekom Biotech.

Each end-user should carry out an analysis for their particular application.

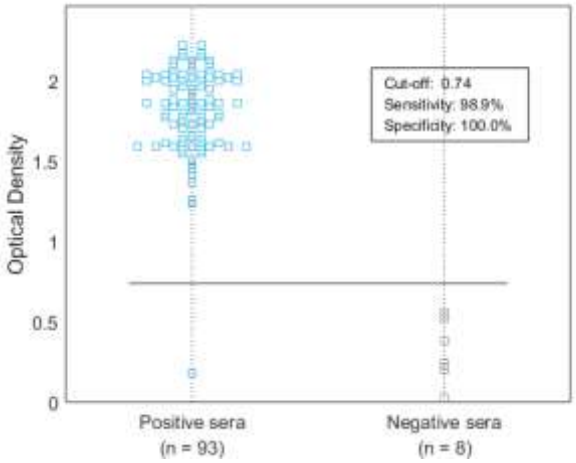

Figure 2. The dot plot graph illustrates the distribution of positive and negative sera by an indirect IgG ELISA with an HBcAg plate coating of 1 µg/ml. Pre-validated sera by HB_SAg (Izasa test) were used in this analysis. The chart shows the optical density at 450/620 nm for positive (blue) and negative (grey) IgG sera.

4. ABSENCE OF PRECIPITATION AFTER A FREEZING AND THAWING CYCLE: ok

LOT SPECIFICATIONS:

1. CONCENTRATION: 1.11 mg/ml

2. TOTAL QUANTITY PER ALIQUOT: 1 mg

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3. TOTAL VOLUME PER ALIQUOT: 0.946 ml

4. SUGGESTED TITER BY ELISA: up to 1:1,110, which corresponds to 1 μg/ml of protein concentration in plates for IgG detection in an indirect ELISA assay.

5. STORAGE: Protein is shipped with dry ice. Upon arrival, it should be aliquoted to avoid repeated freezing and thawing cycles and stored at -20ºC to -80ºC. In order to defrost the protein, maintain the aliquot at 25ºC without shaking to avoid aggregation.

6. TESTED APPLICATIONS: ELISA.

7. POSIBLE APPLICATIONS: WB, DB, Indirect ELISA, positive control in direct ELISA. Where this product has not been tested for use in a particular technique, this does not necessarily exclude its use in such procedures. Suggested working dilutions are given as a guide only. It is recommended that the user titrates.

8. OBSERVATIONS: In some cases, purified proteins run at a molecular weight which is slightly different to the theoretically calculated molecular weight maybe due to the his-tag which can produce a delay in SDS-PAGE. Proteins should be maintained frozen at high concentrations. The dilution to be performed for ELISA assays should be made with a small quantity of protein, the same day of the experiment. In order to defrost the protein, maintain the aliquot at 25ºC without shaking to avoid aggregation. Prior making test dilutions and after defrosting the protein, is recommended to remove possible protein aggregates by centrifuging the stock solution, avoiding alterations in the immobilization of the biomolecule to the solid surface.

RELATED PRODUCTS:

HBeAg.

BIBLIOGRAPHY:

Rolland, D., Gauthier, M., Dugua, J.M., Fournier, C., Delpech, L., Watelet, B., Letourneur, O., Arnaud, M., and M. Jolivet. Purification of recombinant HBc antigen expressed in *Escherichia coli* and *Pichia pastoris*: comparison of size-exclusion chromatography and ultracentrifugation. Journal of Chromatography B, 753 (2001) 51–65.

Gill SC, von Hippel PH. Calculation of protein extinction coefficients from amino acid sequence data. Anal Biochem. 1989 Nov 1;182(2):319-26.

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 recombinant antigens are expressed in non-pathogenic *E. coli* and bacterial integrity is destroyed during purification, the antigen 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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