Novel antibody specific for HBV DNA virus
HB surface antigen glycan isomer (HBsAgGi)

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A monoclonal antibody to *O*-glycosylated PreS2 on M-HBs, which is predominantly expressed by DNA-containing viral particle

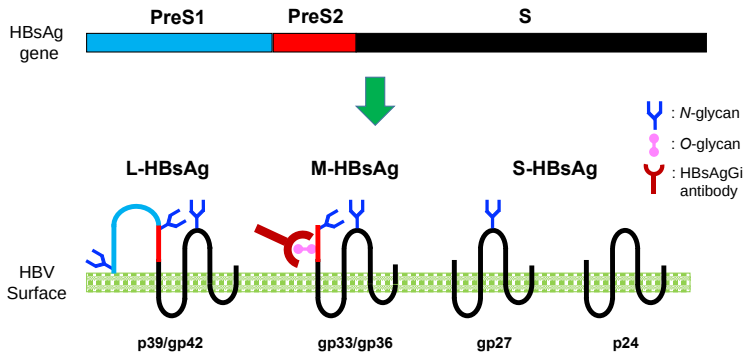

Figure 1: HBsAg and its glycan isomer (HBsAgGi)
HBsAgGi is a monoclonal antibody recognizing *O*-glycosylated PreS2 domain on M-HBs, which is predominantly expressed by DNA-containing viral particle, but not on subviral and empty particles.

HBV envelope protein is composed of three types of surface antigens, S-, M-, and L-HBs, which are produced from an HBsAg gene containing PreS1, PreS2 and S-domains[1]. HBsAgs are heavily glycosylated with *N*-glycan and *O*-glycan[2,3]. Whole glycan structural analyses revealed that PreS2 domain on M-HBs, but not on L-HBs, contains highly conserved *O*-glycosylated site in genotype C (gC)[4]. Compared to traditional HBsAg-testing which recognizes all viral particles, HBsAgGi specifically recognizes infectious HBV particles (DNA virion).

Features:
Binding to intact HBV particles (Immunoprecipitation)
to detect and enrich rare DNA virion from large pool of HBV particle by simple and one-step procedure.
Measuring serum HBV particles (ELISA)
to detect and/or monitor infectious status in chronic hepatitis B patients including virally suppressed population.

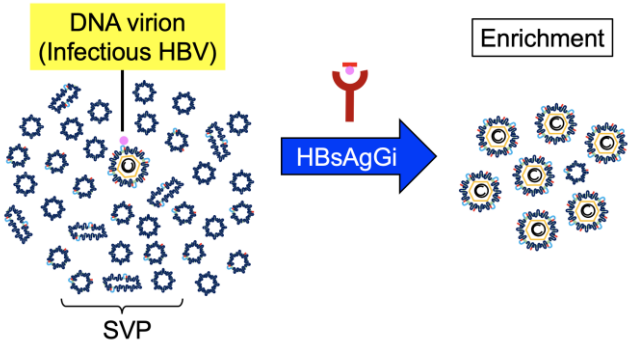

Figure 2: Isolation of rare DNA virion by HBsAgGi
In sera from HBV infected patients, the majority (over 99.9%) of HBV particle is sub viral particle (SVP), which mainly contains S-HBs. In contrast, infectious particle (DNA virion) is less than 0.1% of the whole HBV particles. HBsAgGi can differentiate glycopeptide structure (pink) specific for rare DNA-containing particle from majority of HBV particle.

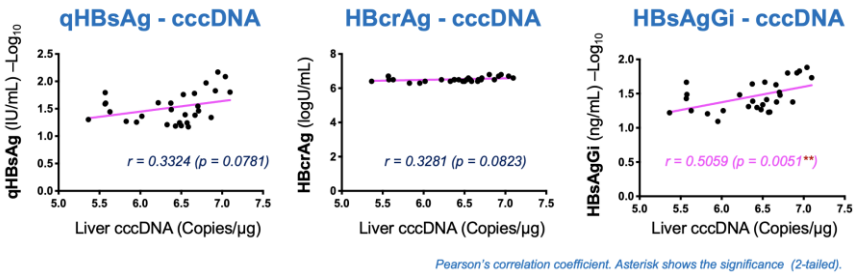

Figure 3: Correlation of liver cccDNA with HBsAgGi (Mouse)
In PhoenixBio (PXB) uPA/SCID chimeric mouse infected with human HBV, serum levels of HBsAgGi were measured by ELISA (n=29). Correlation of liver cccDNA with serum HBsAgGi was superior to that of qHBsAg or HBcrAg in this mouse model (Unpublished data).

References:
1. Schadler and Hildt (2009) Viruses 1:185-209.
2. Schmitt et al. (2004) J Gen Virol 85:2045-2053.
3. Dobrica et al. (2020) Cells 9:1404.
4. Wagatsuma et al. (2018) Anal Chem 90:10196-10203.

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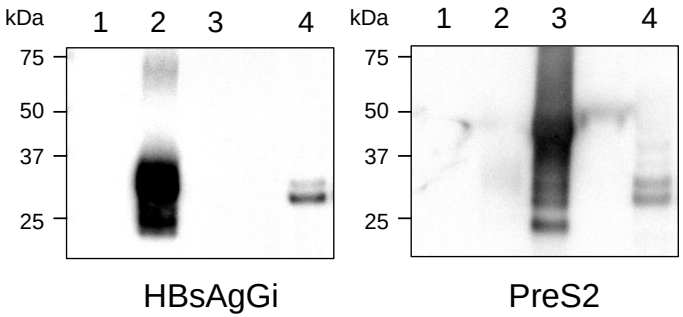
Applications:
1. Western
2. Immunoprecipitation/Separation of HBV particles
3. ELISA
4. HBV infection inhibition assay
5. Immunohistochemistry

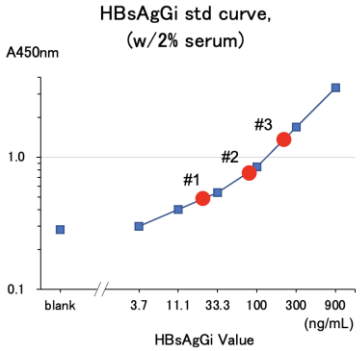
- **Western blotting using PreS2 antibodies**



Left: HBsAgGi-gC
Right: anti-PreS2 antibody
Lane1: S-HBs (CHO) 50 ng
Lane2: M-HBs (HEK293) 50 ng
Lane3: L-HBs (yeast) 50 ng
Lane4: serum (HBV patient) 1 µg
Secondary antibody: HRP -labeled anti-mouse IgG

HBsAgGi-gC (left) detects M-HBs but not L-HBs. On the other hand, a commercially available PreS2 antibody (right) strongly recognizes L-HBs but weakly M-HBs. In human serum of HBV patients, HBsAgGi-gC recognizes M-HBs, while the PreS2 antibody recognizes M-HBs and L-HBs.

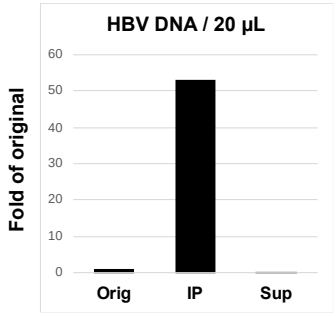
- **ELISA using HBsAgGi-gC coated plate and recombinant M-HBs as a standard sample**



Standard material: M-HBs (3.7 – 900 ng/mL)
Tested samples: sera from HBV patients (2 µL)
Serum #1: HBsAg 6610 IU/mL, HBV DNA 3.1
Serum #2: HBsAg 8612 IU/mL, HBV DNA 0
Serum #3: HBsAg 36600 IU/mL, HBV DNA 8.3
Detection: Biotinylated HBsAgGi-gC and HRP-labeled streptavidin

ELISA plate was coated with HBsAgGi-gC and M-HBs expressed from HEK293 cells was used to obtain standard curve. Human sera of HBV patients (#1-3) were diluted (2 µL serum in 100 µL) and measured on the same ELISA plate. Serum sample (HBV DNA = 0) after NUC treatment was also detected by HBsAgGi-gC.

- **Immunoprecipitation using HBsAgGi-gC**



Original sample (Orig): HBV patient serum (4.1 logIU/20 µL)
IP condition: Biotinylated HBsAgGi-gC 2 µg, Streptavidin-conjugated magnetic beads 10 µL/1 mL TBS-T, 4℃, 16 hours
Quantification of HBV DNA: HBV DNA per 20 µL from precipitated fraction (IP) and supernatant fraction (Sup) was measured by real-time PCR method.

HBV particles were diluted into 1 mL (4.1 logIU/20 µL) and precipitated by HBsAgGi-gC. HBsAgGi-gC could collect the HBV particles containing HBV DNA in this condition.