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

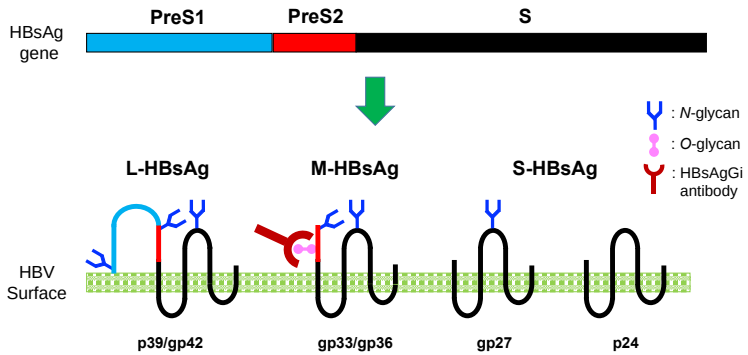

Figure 1: HBsAg and its glycan isomer (HBsAgGi)
HBsAgGi is a monoclonal antibody recognizing *O*-glycosylated PreS2 domain on M-HBs, which is predominantly expressed by DNA-containing viral particle, but not on subviral and empty particles.

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.

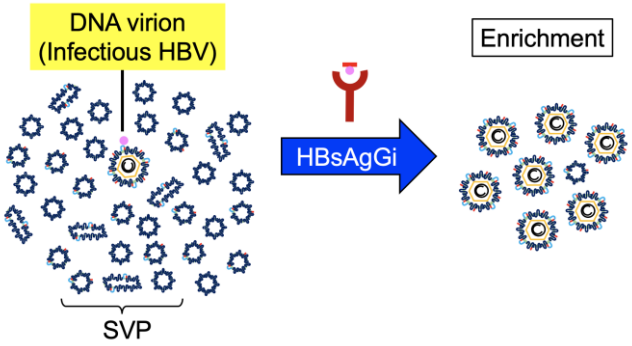

Figure 2: Isolation of rare DNA virion by HBsAgGi
In sera from HBV infected patients, the majority (over 99.9%) of HBV particle is sub viral particle (SVP), which mainly contains S-HBs. In contrast, infectious particle (DNA virion) is less than 0.1% of the whole HBV particles. HBsAgGi can differentiate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

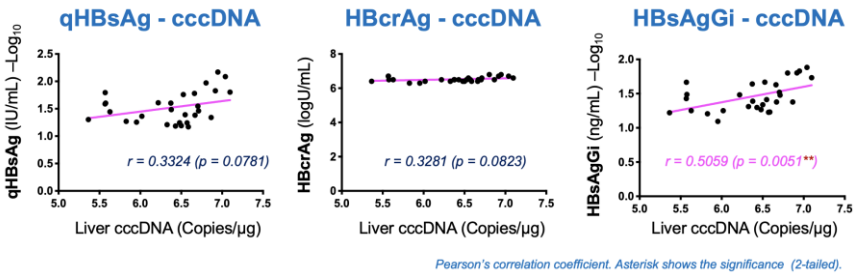

Figure 3: Correlation of liver cccDNA with HBsAgGi (Mouse)
In PhoenixBio (PXB) uPA/SCID chimeric mouse infected with human HBV, serum levels of HBsAgGi were measured by ELISA (n=29). Correlation of liver cccDNA with serum HBsAgGi was superior to that of qHBsAg or HBcrAg in this mouse model (Unpublished 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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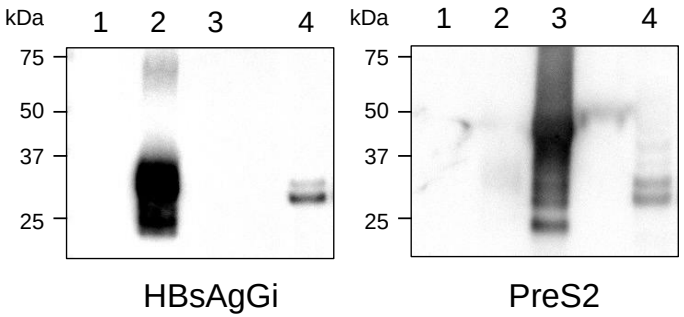
Applications:
1. Western
2. Immunoprecipitation/Separation of HBV particles
3. ELISA
4. HBV infection inhibition assay
5. Immunohistochemistry

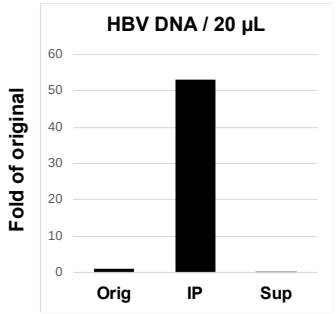
- **Western blotting using PreS2 antibodies**



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.

- **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.

Quantifying infectious HBV particles by HBsAgGi

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An ELISA system using HBsAgGi antibody to detect HBV particles containing HBV DNA or HBV RNA

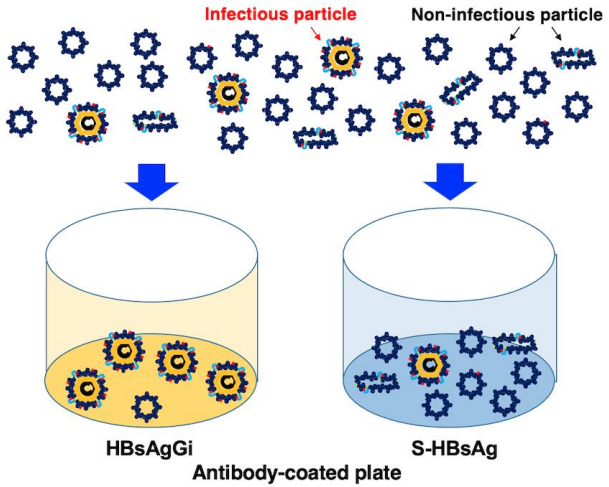
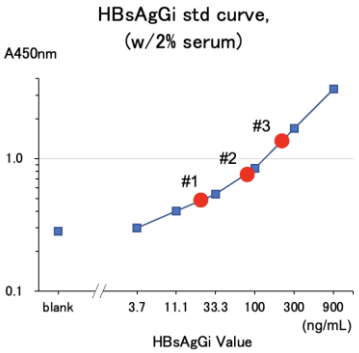


Figure 4: HBsAgGi ELISA can measure HBV DNA particles
Sera of HBV patient mainly contains subviral particles over the HBV DNA particles. S-HBsAg antibody can capture all particles, while HBsAgGi capture infectious HBV particles containing *O*-glycosylated M-HBsAg. Measuring HBV by HBsAgGi will present pathological conditions not obtained by S-HBsAg measurement.

HBV virion is covered by envelope protein (HBsAg). As shown in Figure 1, PreS2 in M-HBsAg is uniquely modified with *O*-glycan in subtype-specific manner[1-3]. Infectious virions contain all three HBsAgs, but non-infectious particles mainly contain S-HBsAg. For the current diagnosis or analysis of HBsAg, antibodies against S-HBsAg are used to measure amount of HBsAg. RCMG's HBsAgGi (HBsAg glycan isomer) is a monoclonal antibody recognizing *O*-glycosylated M-HBsAg that is included in infectious HBV containing HBV DNA[3]. HBV particles captured by HBsAgGi differ from those captured by S-HBsAg antibody. The amount of infectious HBV particles is crucial to monitor pathological condition of the patient. HBsAgGi ELISA is the most convenient method to measure HBV particles containing HBV DNA or HBV RNA.

Features:
Measuring serum HBV particles (ELISA)
- to detect and/or monitor infectious status in chronic hepatitis B patients including virally suppressed population.
- to detect HBV particles containing HBV RNA in patients treated with NUC.
- to detect HBV which are not detected by conventional S-HBsAg antibody caused by mutation(s) in S-domain.

➢ **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.

References:
1. Schmitt et al. (2004) J Gen Virol 85:2045-2053.
2. Dobrica et al. (2020) Cells 9:1404.
3. Angata et al. (2021) Biochim Biophys Acta Gen Subj. 1866:130020