Hepatitis B virus core antigen Ab ELISA Kit

Catalog Number KA0288

96 assays

Version: 02

Intended for research use only

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Introduction

Background

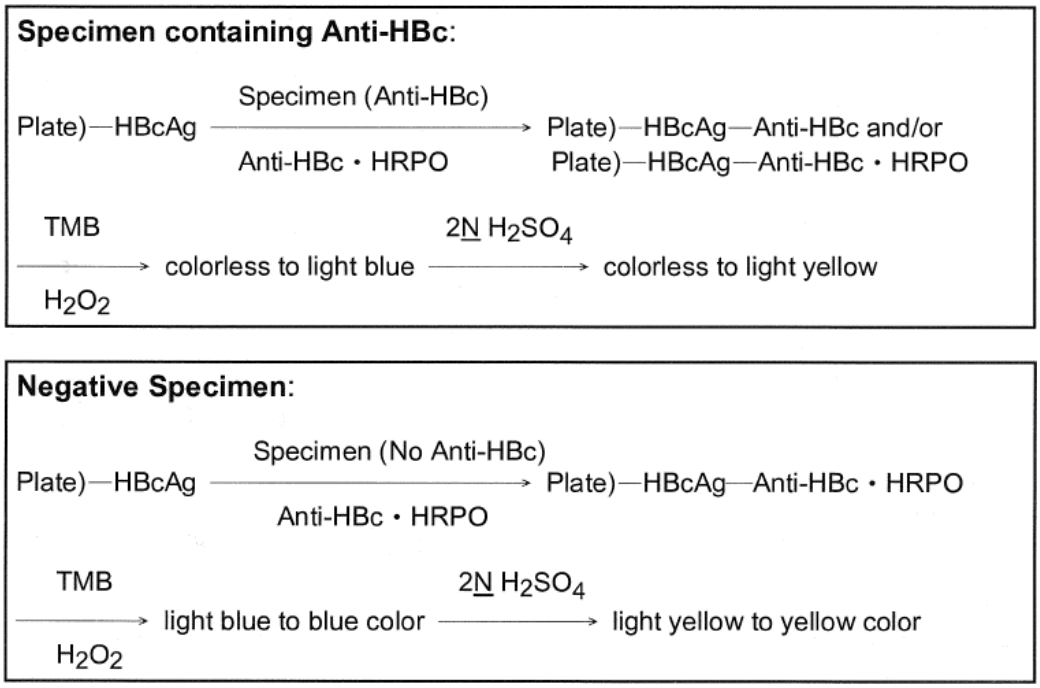
The hepatitis B virus (HBV) consists of an external envelope (HBsAg) and an inner core (HBcAg). In acute HBV infection, antibody to HBcAg (Anti-HBc total) is detectable in serum or plasma shortly before clinical symptoms and slightly after the appearance of HBsAg. In cases in which HBV infection resolves, anti-HBc appears in blood during the window period following loss of HBsAg and prior to the development of antibody to HBsAg (anti-HBs). Anti-HBc is found in acute and chronic hepatitis B patients and also indicates past resolved infection. Therefore, detection of anti-HBc is indicative of exposure to HBV and thus of infection by this virus. Further testing of HBV serological markers is required to define the specific disease state.*1-6

HEPATITIS B VIRUS CORE ANTIGEN ANTIBODY ELISA KIT (TMB) is a fast test for the qualitative detection of the presence of antibodies to HBcAg in serum or plasma (heparin, citrate or EDTA) specimens. The test utilizes HBcAg on microtiter wells and human peroxidase-conjugated Anti-HBc in a competition principle to detect Anti-HBc levels in serum or plasma.
Specimens with absorbance values greater than 1.1 x Cutoff Value are considered NEGATIVE for Anti-HBc.
Specimens with absorbance values less than 0.9 x Cutoff Value are considered POSITIVE for Anti-HBc.
The test has to be repeated in duplicate for specimens with absorbance value within the retest range (Cutoff Value ± 10 %) and interpreted as above.
If the absorbance of any of the specimens retested in duplicate is still within the retest range, it is suggested to test follow-up samples of the patient.

Principle of the Assay

HEPATITIS B VIRUS CORE ANTIGEN ANTIBODY ELISA KIT (TMB) is a solid-phase enzyme immunoassay (ELISA= enzyme-linked immune assay) - based on a competitive principle. The solid phase of the microtiter plate is made of polystyrene wells coated with HBcAg and the liquid phase of human peroxidase conjugated Anti-HBc.
When a serum or plasma specimen containing Anti-HBc is added to the HBcAg-coated wells together with the human peroxidase conjugated Anti-HBc and incubated, a competition will take place for the binding to the HBcAg on the wells. (HBcAg)-(Anti-HBc • Peroxidase) complex and/or (HBcAg)-(Anti-HBc) complex will form on the wells. After washing the microtiter plate to remove unbound material, a solution of TMB substrate is added to the wells and incubated. Due to the competitive principle a color develops inversely proportional to the amount of Anti-HBc bound to HBcAg deriving from the specimen. The Peroxidase-TMB reaction is stopped by addition of sulfuric acid. The optical density of developed color is read with a suitable photometer at 450 nm with a selected reference wavelength within 620 to 690 nm*8.

The above test principle is shown also in the following figure.



General Information

Materials Supplied

List of component

Component	Description	Amount
HBcAg Plate	Microtiter plate coated with HBcAg.	1 plate
Anti-HBc · Peroxidase Solution	Anti-HBc (human) · Peroxidase conjugate dissolved in buffer with protein stabilizers. Preservatives: 0.003% Gentamycin and 0.01% Thimerosal.	5 ml
Anti-HBc Positive Control	Anti-HBc positive serum in buffer with protein stabilizers. Preservatives: 0.003% Gentamycin and 0.01% Thimerosal.	1 ml
HB Negative Control	Serum non-reactive for HBV markers. Preservatives: 0.003% Gentamycin and 0.01% Thimerosal.	1.5 ml
TMB Substrate Solution A	0.6 mg/ml of 3,3',5,5'-tetramethylbenzidine (TMB) in an organic base.	10 ml
TMB Substrate Solution B	Citrate acid buffer containing 0.03% H_2O_2.	10 ml
Conc. Washing Solution D (20x)	Concentrated phosphate buffer with Tween-20	50 ml
2N H_2SO_4	2N H_2SO_4 (Sulfuric Acid)	10 ml

Storage Instruction

- ✓ The kit must be stored at 2-8℃. Do not freeze.
- ✓ Strips of the plate should be used within one month after open the original aluminum foil bag. The unused strips should be kept in the aluminum foil bag and tapped the opening tightly.
- ✓ Return the reagents to 2-8℃ immediately after use.
- ✓ Washing Solution D (20X) Concentrate should be stored at room temperature to avoid crystallization. If the crystal has been precipitated before use, warm up the solution in a 37℃ water bath till crystal dissolved.

Materials Required but Not Supplied

- ✓ 50µl, 100µl micropipettes and tips are needed
- ✓ Incubator or water bath with temperature control at +37°C

- ✓ Plate washing equipment.
- ✓ ELISA microwell reader:
  Dual wavelength 450nm with 620-690nm as reference wavelength*8, bandwidth 10nm.
- ✓ Fully automatic EIA micro-plate analyzer is optional. User should validate the automatic EIA micro-plate analyzer in combination with the kit.

Precautions for Use

- Procedural Guidelines

- ✓ This kit is for medical technicians or physicians used only.
- ✓ This reagent kit is for in vitro diagnosis only.
- ✓ Bring all kit reagents and samples to room temperature (20-30℃) and mix gently before use.
- ✓ Do not use kit beyond its expiration date.
- ✓ Do not interchange reagents between different lots.
- ✓ Reagents must be protected from microbial contamination.
- ✓ The positive and negative control sera have been inactivated, however, for safety reason, they must be treated as infectious material.
- ✓ Do not smoke or eat in areas where specimens or reagents are handled.
- ✓ Do not pipette by mouth.
- ✓ Wear gloves when handling reagents or specimens, and wash hands thoroughly afterwards.
- ✓ Infectious specimens and nonacid containing spills should be wiped up thoroughly with 5% sodium hypochlorite.
- ✓ All waste materials should be properly disinfected before disposal. Both liquid and solid waste should be autoclaved for at least 1 hour at 121℃. Solid waste can also be incinerated. Non-acidic liquid waste can be treated with sodium hypochlorite diluted to a final concentration of 1%. Liquid waste containing acid must be neutralized before similar treatment and should stand for 30 minutes to obtain effective disinfection.
- ✓ TMB substrate solution A contains dimethyl sulfoxide, an irritant to skin and mucous membranes. Avoid contact of TMB substrate solution and sulfuric acid with skin and mucous membranes.

- Limitations and Interferences

- ✓ This reagent kit is to be used for un-pooled human serum or plasma samples only.
- ✓ The reagent kit has not been validated for use with cadaveric samples.
- ✓ Non-repeatable false positive results may be obtained with any enzyme immunoassay kit, largely due to technical error either on the part of the operator or malfunction of apparatus used.
- ✓ Potential interfering substances:
  Potential interfering samples, i.e. samples with hyperlipemia, hemolysis, hyper-bilirubinemia, and samples with monoclonal immunoglobulin components, samples containing elevated levels of

autoimmune antibodies (rheumatoid factor-RF, antinuclear antibodies-ANA, or anti-mitochondrial antibodies-ANA) did not affect the test result with HEPATITIS B VIRUS CORE ANTIGEN ANTIBODY ELISA KIT (TMB).

- ✓ The anticoagulants heparin, EDTA and sodium citrate have no influence on the specificity of HEPATITIS B VIRUS CORE ANTIGEN ANTIBODY ELISA KIT (TMB) and can be used to obtain plasma samples for analysis with the Anti-HBc Total kit.

Assay Protocol

Reagent Preparation

- Washing solution

Dilute Washing Solution D (20x) Concentrate with distilled or de-ionized water to 1:20 dilution. Do not use tap water.

Plate Washing Procedure

A. AUTOMATIC OR SEMI-AUTOMATIC PLATE WASHER

Any commercial automatic microplate washer or other liquid aspirating/dispensing devices can be used for washing purpose. The user should test the devices to determine the proper volume of water and wash cycles to insure proper washing. We suggested 6 wash cycles with at least 350 μ l per well per wash and soaking for 10 seconds is necessary.

B. MANUAL PLATE WASH

Cover the reaction plate with an absorbent paper. Invert the plate and allow the liquid absorb onto the absorbent paper, then return the plate back to upright position. Fill each well with 350μl of washing buffer. Aspirate the water after soaking 10 seconds. Repeat this procedure 6 times. Blot dry by inverting the plate and tapping firmly onto absorbent paper. All residual washing buffer should be blotted dry.

WARNING: Improper washing can cause false results.

Sample Preparation

- Specimen Collection and Preparation for Analysis

✓ Either serum or plasma can be used with this diagnostic kit. Whole blood specimens should be separated as soon as possible in order to avoid hemolysis. Also, clots must be removed.

✓ Specimens must be stored at 2-8℃ and avoided heat-inactivation to minimize deterioration. For long-term storage, they should be frozen below -20℃. Storage in self-defrosting freezer is not recommended.

✓ Avoid multiple freeze-thaw procedures.

✓ Frozen specimens must be thawed thoroughly and mixed before test.

✓ Specimens must not contain any sodium azide, which inhibits the peroxidase activity.

Assay Procedure

1. Bring all reagents and specimens to room temperature (20-30℃) before assay. Adjust a water bath or incubator to 37±1℃.
2. Reserve 2 wells for blanks. Add 50µl of each control or specimen to appropriate wells of reaction plate (3 Negative Controls and 2 Positive Controls).

NOTE: Use a new pipette tip for each sampling to avoid cross contamination.

3. Add 50µl of Anti-HBc・Peroxidase Solution to each well except 2 blanks.
4. Gently tap the plate.
5. Remove the protective backing from the Adhesive Slip and press it on the reaction plate, so that it is tightly sealed.
6. Incubate the reaction plate in a 37±1℃ water bath for 1 hour.
7. At the end of the incubation period, remove and discard the Adhesive Slip and wash plate by following "PLATE WASHING PROCEDURES".
8. Choice one of the following two methods for color development:

NOTE: TMB Substrate Solution A should be colorless to light blue, otherwise, should be discarded. The mixture of TMB Substrate Solution A and B should be used within 30 minutes after mix. The mixture should be avoided from intense light.

A. Mix equal volume of TMB Substrate Solution A and B in a clean container immediately prior to use. Add 100µl of the mixture solution to each well including 2 blank wells.
B. Add 50µl of TMB Substrate Solution A first, then add 50µl of TMB Substrate Solution B into each well including 2 blanks. Mix well gently.

9. Cover the plate with Black Cover and incubate at room temperature for 30 minutes.
10. Stop the reaction by adding 100µl 2N H_2SO_4 to each well including 2 blanks.
11. Determine absorbance of Controls and test specimens within 15 minutes with a precision spectrophotometer at 450nm or 450/650nm (450nm reading wavelength with 650nm reference wavelength). Use the lighter color of two blank wells to blank the spectrophotometer.

NOTE: The color of the blank should be colorless to pale yellow, otherwise, the test must be repeated.

Data Analysis

Calculation of Results

Calculation of the NCx (Mean Absorbance of Negative Control)

Example:

Sample No.	Absorbance
1	0.939
2	0.944
3	0.925

NCx = (0.939 + 0.944 + 0.925) / 3 = 0.936

NCx must be≥ 0.4, otherwise, the test run is invalid.

Calculation of the PCx (Mean Absorbance of Positive Control)

Example:

Sample No.	Absorbance
1	0.068
2	0.052

PCx = (0.068 + 0.052) /2 = 0.060

PC x must be ≤ 0.1, otherwise, the test run is invalid.

Calculation of the N - P Value

N - P = NC x – PC x

Example:

N – P = 0.936 – 0.060 = 0.876

N - P Value must be ≥ 0.3, otherwise, the test run is invalid.

Calculation of the Cutoff Value

Cutoff Value = 0.4 NCx + 0.6 PCx

Example:

Cutoff Value = (0.4 x 0.936) + (0.6 x 0.060) = 0.410

Calculation of the Retest Range

Retest Range = Cutoff Value ± 10%

Example: Cutoff Value = 0.410

Retest Range = (0.410 - 0.041) to (0.410 + 0.041) = 0.369 to 0.451

Note:

If the signal/cut-off ratio is within Retest Range (0.9-1.1 x cutoff), the test must be repeated in duplicate and

interpreted as above. If both results are non-reactive the final result is non-reactive, if both results are reactive the final result is reactive. Any other combination is an indeterminate result. Testing of follow up samples and other hepatitis B serological markers should be taken into account in case of an indeterminate result.

Performance Characteristics

- Diagnostic Specificity

Negative specimens/Specimens used to evaluate the specificity

True Negative Samples		HEPATITIS B VIRUS CORE ANTIGEN ANTIBODY ELISA KIT (TMB)
Type of sample	Number of samples	No. negative samples
Blood donor samples	5020	5010
Samples from hospitalized persons	200	200
Samples contain potential interfering factors	97	97
Samples with added possible interfering factors	12	11
Samples with different anticoagulants	48	48
Total	5377	5366
Diagnostic Specificity	----------	5366/5377 = 99.8%

Potential interferences with HEPATITIS B VIRUS CORE ANTIGEN ANTIBODY ELISA KIT (TMB) assay were investigated.

For each potential interfering substance, at least two serum samples containing different amounts of the potentially interfering substance were mixed in fixed ratios of 10 + 0; 7 + 3; 5 + 5; 3 + 7; 0 + 10 with other serum samples containing increased Anti-HBc Total levels but no interfering factors. The neat samples as well as the mixtures were analyzed.

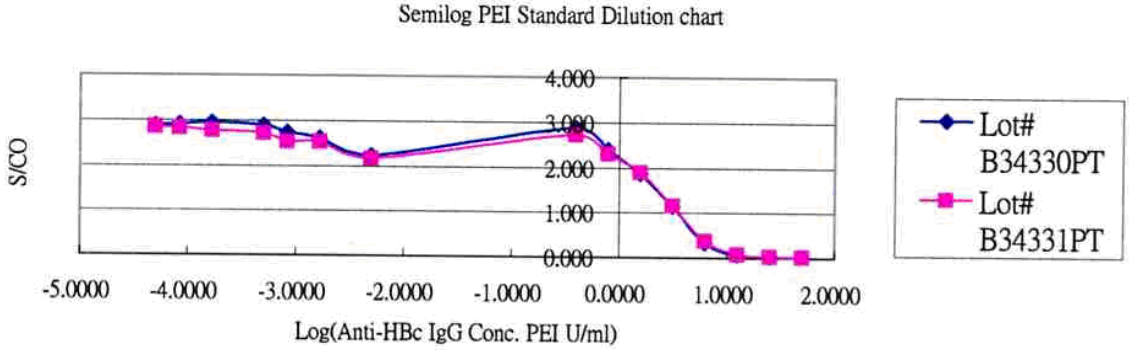
In particular the specificity study included:

- ✓ lipemic (turbid) samples (hyperlipidemia) before and after high speed centrifugation
- ✓ hemolytic samples or hemolysate
- ✓ icteric samples (=hyperbilirubinemia)
- ✓ samples with monoclonal immunoglobulin components (hyperimmunoglobulinemia)
- ✓ samples containing elevated levels of autoimmune antibodies (rheumatoid factor - RF, antinuclear antibodies –ANA, or antimitochondrial antibodies-AMA).

No interferences were detected with both used lots. Neither the type of anticoagulant had an influence on both tested lots of HEPATITIS B VIRUS CORE ANTIGEN ANTIBODY ELISA KIT (TMB).

- Analytical Sensitivity and Linearity

To evaluate the sensitivity of HEPATITIS B VIRUS CORE ANTIGEN ANTIBODY ELISA KIT (TMB) serial dilutions of the Standard Material for Anti-HBc Total of Paul Ehrlich Institute (PEI) (Langen, Germany) (100 PEI U/ml) were used.



For Lot# B34330PT: Linearity ,R=		-0.994	
For Lot# B34331PT: Linearity, R =		-0.991	
Worst Case: Linearity, R =		-0.991	
Lot#	A	B	
B34330PT	2.1397	-2.0009	
B34331PT	2.0757	-1.8801	
Lot#	X=(Y-A)B		
B34330PT	Detection Limit =	1.858	PEI U/ml
B34331PT	Detection Limit =	1.869	PEI U/ml
Worst Case	Detection Limit =	1.869	PEI U/ml

The analytical sensitivity (detection limit) was defined as the lowest concentration which can be detected, i.e. at CO/S≥1.1 (i.e. S/CO≤0.9) calculated by using the linear regression function.

- Diagnostic Sensitivity

✓ HBV infected individuals

435 HBV-positive samples were measured with both HEPATITIS B VIRUS CORE ANTIGEN ANTIBODY ELISA KIT (TMB) and the reference assay. The diagnostic sensitivity for the ABNOVA assay was 100% as it was for the reference assay.

✓ Commercial seroconversion panels

Eight commercially available seroconversion panels consisting of follow-up samples which were collected at weekly or monthly intervals from patients suffering from acute hepatitis B, have been used. The panels were obtained from Boston Biomedica Inc., BBI; West Bridgewater, MA USA (PHM 933, PHM 934 and PHM 935A); Pyramid-Profile Diagnostics, Sherman Oaks, CA, USA (RP 009, RP 016 and RP 017) and NABI, Boca Roton, FL, USA (SB 411 and SB 413). All the panels have been characterized for HBV-specific serological markers (anti-HBs, anti-HBc IgM, anti-HBc-Total, and HBsAg).

By testing of the seroconversion panels ABNOVA HEPATITIS B VIRUS CORE ANTIGEN ANTIBODY ELISA KIT (TMB) detected Anti-HBc Total one bleed earlier in the NABI panel RP-009 and the reference assay detected the Anti-HBc Total two bleeds earlier in the BBI Panel 935A and one bleed earlier in the NABI panel RP-017. In the other 5 panels ABNOVA HEPATITIS B VIRUS CORE ANTIGEN ANTIBODY ELISA KIT (TMB) and the reference assay detected

Anti-HBc Total in the same bleed.

In summary there was no significant difference between the ABNOVA HEPATITIS B VIRUS CORE ANTIGEN ANTIBODY ELISA KIT (TMB) assay and the reference assay.

- Precision

✓ Intra-run repeatability

For determination of intra-assay (within-run) precision, the Positive Control provided with the test kit and two patient serum samples with different Anti-HBc Total titer (slightly above the cutoff level and at medium level) were analyzed in replicates of 20 in a single “run” over 3 days. The CVs were in an acceptable range for both tested lots.

Item tested	Sample size	Precision
Positive Control	N = 20	CV ≤ 12.68%
Patient Serum #1	N = 20	CV ≤ 10.62%
Patient Serum #2	N = 20	CV ≤ 16.72%

✓ Inter-run reproducibility

Item tested	Sample size	Precision
Positive Control	N = 60	CV ≤ 7.44%
Patient Serum #1	N = 60	CV ≤ 8.81%
Patient Serum #2	N = 60	CV ≤14.67%

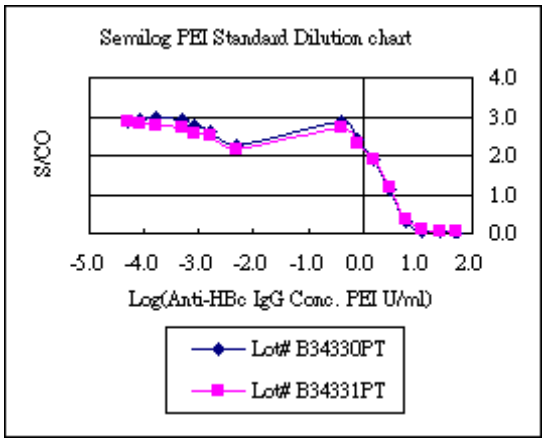
- Traceability

Concentration of Positive Control of HEPATITIS B VIRUS CORE ANTIGEN ANTIBODY ELISA KIT (TMB) referred to the PEI Anti-HBc Total Reference Material = 70 PEI U/ml ± 30%

- Antibody Excess/High-Dose Hook Effect

The effect of antibody excess was tested by consecutive dilution of a standard material having very high Anti-HBc levels (PEI Anti-HBc Total Reference Material).

Semilog Dilution Chart



The Semilog PEI Standard Dilution chart illustrates that an antigen/antibody excess is not occurring also because of the reverse reaction used in this assay format.

An antigen/antibody excess will not influence the reactive/non-reactive interpretation.

Resources

Troubleshooting

If the result cannot be reproduced, a preliminary troubleshooting should be performed by checking the possibilities listed below:

- ✓ Improper washing procedure.
- ✓ Contaminated with positive specimen.
- ✓ Wrong volume of sample, conjugate or substrate.
- ✓ Contamination of well rim with conjugate.
- ✓ Improper specimen such as hemolyzed serum or plasma, specimen containing precipitate and specimen not thoroughly mixed before use.
- ✓ Wrong incubation time or temperature.
- ✓ Obstructed or partial obstructed washer aspirate/dispense head and needles.
- ✓ Insufficient aspiration.

References

1. Aach RD, Grisham JW, Paker CW. Detection of Australia antigen by radioimmunoassay. Proc Natl Acad Sci. USA 1971; 68:1056-1060.
2. Kim CY, Tikes JG. Purification and biophysical characterization of hepatitis antigen. J Clin Invest. 1973; 52:1176-1186.
3. Hoofnagle JH, Gerety RJ, Barker LF, Antibody to hepatitis B virus core in man. Lancet. 1973: 2(7834): 869-873.
4. Barker LF, Almeida JD, Hoofnagle JH, et al. Hepatitis B core antigen: immunology and electron microscopy. J Virol. 1974 Dec;14:1552-1558.
5. Hoofnagle. JH. Gerety, RJ.. Ni, LY.. Barker, LF. Antibody 10 Hepatitis B core antigen: A sensitive Indicator of hepatitis B virus replication. New Engl J Med. 1974; 290:1336-1340.
6. Niermeijer, P., Gips, C. H., Huizenga, J. R. et al. IgM Anti-HBc as a marker of persistent and IgG anti-HBc as a marker of past hepatitis B infection. A longitudinal study over 5 years. Acta Hepato-Gastroenterol 1978; 25: 360–364.
7. Shikata T, Karasawa T, Abe K, et al. Incomplete inactivation of hepatitis B virus after heat treatment at +60°C for 10 hours, J. Infect. Dis. 1978; 138:242-244.
8. The reference wavelength of spectrometer can be 620nm to 690nm. However, user should validate the photometer in combination with this kit before use.

Plate Layout

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11								
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