HK3002

Human C3bBbP

Edition 05-25

ELISA KIT
PRODUCT INFORMATION & MANUAL

Read carefully prior to starting procedures!
For use in laboratory research only
Not for clinical or diagnostic use

info@hycultbiotech.com ● www.hycultbiotech.com

Note that this user protocol is not lot-specific and is representative for the current specifications of this product. Please consult the vial label and the Certificate of Analysis for information on specific lots. Also note that shipping conditions may differ from storage conditions.

For research use only. Not for use in or on humans or animals or for diagnostics. It is the responsibility of the user to comply with all local/state and federal rules in the use of this product. Hycult Biotech is not responsible for any patent infringements that might result from the use or derivation of this product.

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1. INTENDED USE

The human C3bBbP ELISA kit is to be used for the *in vitro* quantitative determination of C3bBbP in serum and plasma samples. This kit is intended for laboratory research use only and is not for use in diagnostic or therapeutic procedures.

The analysis should be performed by trained laboratory professionals.

2. INTRODUCTION

The complement system is a vital component of innate immunity, serving initially as the first line of defense against pathogens. It consists of three activation pathways: the classical, lectin, and alternative pathways, all converging at the activation of complement protein C3. The alternative pathway is unique in its ability to amplify immune responses via the formation of the C3 convertase complex, C3bBb, which plays a crucial role in pathogen clearance and immune surveillance.

C3bBbP, also known as the properdin-stabilized C3 convertase, is an extended form of C3bBb. Properdin, the only known positive regulator of complement activity, binds to C3bBb, increasing its stability and prolonging its function. This stabilization enhances the cleavage of C3 into C3a and C3b, leading to a powerful amplification loop that ensures rapid immune responses. However, dysregulation of this mechanism can contribute to pathological conditions.

Elevated levels of C3bBbP have been implicated in complement-mediated diseases, including C3 glomerulopathy (C3G) and atypical hemolytic uremic syndrome (aHUS). These disorders are characterized by excessive complement activation, leading to uncontrolled inflammation and tissue damage. Additionally, the presence of nephritic factors (autoantibodies that stabilize C3bBbP) can further disrupt complement homeostasis, exacerbating disease progression.

The detection of C3bBbP serves as a potential diagnostic biomarker for alternative pathway complement-associated diseases. Its measurement helps to assess alternative pathway activity, providing valuable insights into disease mechanisms and possibly guiding complement-targeted therapies. As complement-directed treatments continue to progress, the role of C3bBbP is becoming increasingly significant.

3. KIT FEATURES

- Working time of 1¼ hours.
- Minimum concentration which can be measured is 0.40 AU/ml.
- Measurable concentration range of 0.40 to 25 AU/ml.
- Working volume of 100 µl/well.

Cross-reactivity

Potential cross-reacting proteins detected in the Human C3bBbP ELISA:

Cross reactant	Reactivity
C3	Negative
C3a	Negative
C3b / iC3b	Negative
C3c	Negative
Factor B	Negative
Factor Ba	Negative
Factor Bb	Negative
Properdin	Negative

Table 1

Potential cross-reactive species detected in the C3bBbP ELISA:

Cross reactant	Reactivity
Monkey (Non-human primate)	Strong
Mouse	Strong
Rat	Weak
Pig	Weak

Table 2

Cross-reactivity for other species or proteins/peptides has not been tested.

4. PROTOCOL OVERVIEW

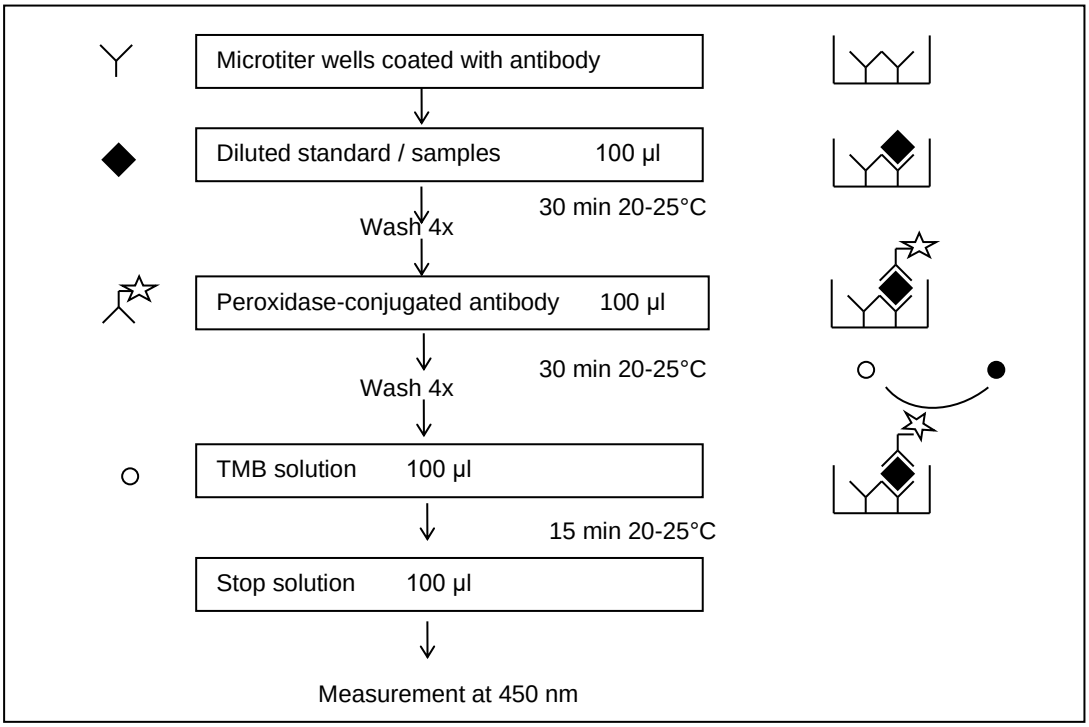

Figure 1

- The human C3bBbP ELISA is a ready-to-use solid-phase enzyme-linked immunosorbent assay based on the sandwich principle with a working time of 1 hour and 15 minutes.
- The efficient format of a plate with twelve disposable 8-well strips allows free choice of batch size for the assay.
- Samples and standards are incubated in microtiter wells coated with antibodies recognizing C3bBbP.
- Peroxidase-conjugated antibody will bind to the captured C3bBbP.
- Peroxidase-conjugate will react with the substrate, tetramethylbenzidine (TMB).
- The enzyme reaction is stopped by the addition of oxalic acid.
- The absorbance at 450 nm is measured with a spectrophotometer. A standard curve is obtained by plotting the absorbance (linear) versus the corresponding concentrations of the C3bBbP standards (log).
- The C3bBbP concentration of samples, which are run concurrently with the standards, can be determined from the standard curve.

5. KIT COMPONENTS AND STORAGE INSTRUCTIONS

Kit component	Cat.#	Quantity HK3002-01	Quantity HK3002-02	Color code
Wash buffer 20x	WB21	1 vial (60 ml)	1 vial (60 ml)	Colorless
Dilution buffer 10x	DB104	1 vial (60 ml)	1 vial (60 ml)	Green
Standard		2 vials, lyophilized	4 vials, lyophilized	White
Peroxidase-conjugated antibody		1 vial, 1 ml lyophilized	2 vials, 1 ml lyophilized	Blue
TMB substrate	TMB050/100	1 vial, (11 ml)	1 vial, (22 ml)	Brown
Stop solution	STOP110	1 vial, (22 ml)	1 vial, (22 ml)	Red
12 Microtiter strips, pre-coated		1 plate	2 plates	
Certificate of Analysis		1	1	
Manual		1	1	
Data collection sheet		1	2	

Table 3

- Upon receipt, store individual components at 2 - 8°C. Do not freeze.
- Do not use components beyond the expiration date printed on the kit label.
- The standard and detection antibody in lyophilized form are stable until the expiration date indicated on the kit label, if stored at 2 - 8°C.
- The exact amount of the standard is indicated on the label of the vial and the Certificate of Analysis.
- The standard is single use. After reconstitution the standard cannot be stored.
- Upon receipt, foil pouch around the plate should be vacuum-sealed and unpunctured. Any irregularities to aforementioned conditions may influence plate performance in the assay.
- Return unused strips immediately to the foil pouch containing the desiccant pack and reseal along the entire edge of the zip-seal. Quality guaranteed for one month if stored at 2 - 8°C.

Materials required but not provided

- Calibrated micropipettes and disposable tips.
- Distilled or de-ionized water.
- Plate washer: automatic or manual.
- Polypropylene tubes.
- Calibrated ELISA plate reader capable of measuring absorbance at 450 nm.
- Centrifuge for 1 ml tubes.

6. WARNINGS AND PRECAUTIONS

- This product is intended for research purposes only and not for use in diagnostic or therapeutic procedures.
- Only qualified personnel trained in laboratory procedures should handle this kit.
- Under no circumstances should sodium azide be added to any component as a preservative.
- Refrain from using kit components beyond their expiration date.
- To ensure accuracy, do not interchange reagents from different kits or lots. Each kit and lot is calibrated as a complete unit; use only the reagents supplied by the manufacturer.
- The assay has been optimized for the indicated standard range. Alterations to the standard range are not recommended.
- Exercise caution when opening vials as they are under vacuum.
- Do not ingest any of the kit components.
- Kit reagents contain 2-chloroacetamide as a preservative. 2-Chloroacetamide is harmful in contact with skin and toxic if swallowed. In case of accident or if you feel unwell, seek medical advice immediately.
- The TMB substrate is light sensitive, keep away from bright light. The solution should be colorless until use.
- The stop solution contains 2% oxalic acid and can cause irritation or burns to respiratory system, skin and eyes. Direct contact with skin and eyes should be strictly avoided. If contact occurs, rinse immediately with plenty of water and seek medical advice.
- Deviations from the specified incubation times, temperatures, or pipetting volumes may result in inaccurate results.
- Do not reuse microwells or pour reagents back into their bottles once dispensed.
- Handle all biological samples as potentially hazardous and capable of transmitting diseases.
- Hemolyzed, hyperlipemic, heat-treated or contaminated samples may give erroneous results.
- Use polypropylene tubes for preparation of standard and samples. Do not use polystyrene tubes or sample plates.
- The standard is of human origin. It has been tested for various viruses and found negative. Since no test method can offer complete assurance that infectious agents are absent, this reagent should be handled as any potentially infectious human serum or blood specimen. Handle all materials in contact with this reagent according to guide-lines for prevention of transmission of blood-borne infections.

7. SAMPLE PREPARATION

Human C3bBbP can be measured in plasma and serum samples. The recommended sample medium is EDTA plasma. All samples should be kept on ice during preparation and should be immediately assayed after thawing to avoid decay of C3bBbP.

Collection and handling

Serum or plasma

Collect blood using normal aseptic techniques. Blood samples should be kept on ice. If serum is used, separate serum from blood after clotting at room temperature within one hour by centrifugation (1500xg at 4°C for 15 min). Transfer the serum to a fresh polypropylene tube.

If plasma is used, separate plasma from blood within 20 minutes after blood sampling by centrifugation (1500xg at 4°C for 15 min). Transfer the plasma to a fresh polypropylene tube.

Most reliable results are obtained if EDTA plasma is used.

Storage

Store samples below -20°C, preferably at -70°C in polypropylene tubes. Storage at -20°C can affect recovery of C3bBbP. Use samples immediately after thawing. Avoid multiple freeze-thaw cycles which may cause loss of C3bBbP and give erroneous results. Do not use hemolyzed, hyperlipemic, heat-treated or contaminated samples. Before performing the assay, samples should be mixed gently. Prepare all samples (controls and test samples) prior to starting the assay procedure. Avoid foaming.

Dilution procedures

Serum or plasma samples

C3bBbP can be measured accurately if serum or plasma samples are diluted at least 10x with supplied dilution buffer in polypropylene tubes. The optimal sample dilution should be determined for each specific sample set/study. It is recommended to use EDTA plasma samples for obtaining the most reliable results.

Comment regarding recommended sample dilution

The mentioned dilution for samples is a minimum dilution and should be used as a guideline. The recovery of C3bBbP from an undiluted sample is not 100% and may vary from sample to sample. When testing less diluted samples it is advisable to run recovery experiments to determine the influence of the matrix on the detection of C3bBbP. Do not use polystyrene tubes or sample plates for preparation or dilution of the samples.

Guideline for dilution of samples

Please see table 4 for recommended sample dilutions. Volumes are based on a total volume of at least 250 µl of diluted sample, which is sufficient for one sample in duplicate in the ELISA. For dilution of samples we recommend to use at least 10 µl of sample.

	Dilution	Pre-dilution	Amount of sample or pre-dilution required	Amount of dilution buffer required
1.	10x	Not necessary	25 µl (sample)	225 µl
2.	20x	Not necessary	15 µl (sample)	285 µl
3.	50x	Not necessary	10 µl (sample)	490 µl
4.	100x	Not necessary	10 µl (sample)	990 µl
5.	500x	Recommended: 10x (see nr.1)	10 µl (pre-dilution)	490 µl
6.	1000x	Recommended: 10x (see nr.1)	10 µl (pre-dilution)	990 µl
7.	2000x	Recommended: 20x (see nr.2)	10 µl (pre-dilution)	990 µl
8.	5000x	Recommended: 50x (see nr.3)	10 µl (pre-dilution)	990 µl

Table 4

8. REAGENT PREPARATION

Allow all the reagents to equilibrate to room temperature (20 – 25°C) prior to use. Return to proper storage conditions immediately after use.

Wash buffer

Prepare wash buffer by mixing 60 ml of 20x wash buffer with 1140 ml of distilled or de-ionized water, which is sufficient for 2 x 96 tests. In case less volume is required, prepare the desired volume of wash buffer by diluting 1 part of the 20x wash buffer with 19 parts of distilled or de-ionized water.

Dilution buffer

Prepare dilution buffer by mixing 60 ml of the 10x dilution buffer with 540 ml of distilled or de-ionized water, which is sufficient for 2 x 96 tests. In case less volume is required, prepare the desired volume of dilution buffer by diluting 1 part of the 10x dilution buffer with 9 parts of distilled or de-ionized water. Concentrated dilution buffer may contain crystals. In case the crystals do not disappear at room temperature within one hour, concentrated dilution buffer can be warmed up to 37°C. Do not shake the solution.

Standard solution

The standard is reconstituted by pipetting the amount of dilution buffer mentioned on the CoA in the standard vial. Use the standard vials as Tube 1 in Figure 2. Prepare each C3bBbP standard in polypropylene tubes on ice by serial dilution of the reconstituted standard with dilution buffer as shown in Figure 2*. After reconstitution the standard cannot be stored for repeated use.

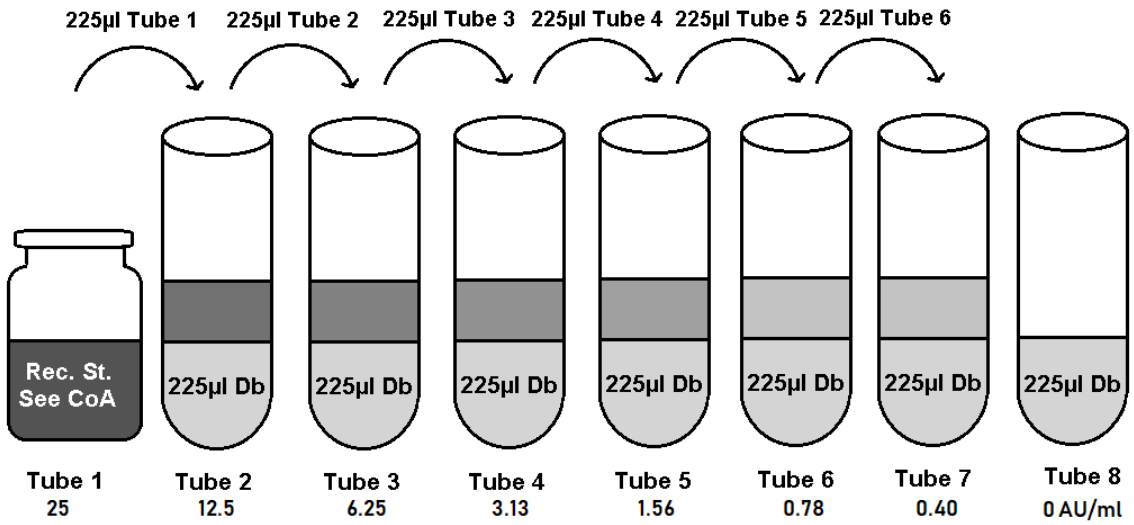

Figure 2

*) CoA: Certificate of Analysis, St: Standard, Db: Dilution buffer

Peroxidase-conjugated antibody

The peroxidase-conjugated antibody is reconstituted by pipetting 1 ml distilled or de-ionized water. Dilute the reconstituted 1 ml detection antibody with 11 ml dilution buffer, which is sufficient for 1 x 96 tests. In case less volume is required, prepare the desired volume of peroxidase-conjugated antibody by diluting 1 part of the reconstituted vial with 11 parts of dilution buffer.

9. ELISA PROTOCOL

Bring all reagents to room temperature (20 - 25°C) before use.

1. Determine the number of test wells required, put the necessary microwell strips into the supplied frame, and fill out the data collection sheet. Return the unused strips to the storage bag with desiccant, seal and store at 2 - 8°C.
2. Transfer 100 µl in duplicate of standard, samples, or controls into appropriate wells. Do not touch the side or bottom of the wells.
3. Cover the tray and tap the tray to eliminate any air bubbles. Be careful not to splash liquid onto the cover.
4. Incubate the strips or plate for 30 minutes at room temperature.
5. Wash the plates 4 times with wash/dilution buffer using a plate washer or as follows*:
   a. Carefully remove the cover, avoid splashing.
   b. Empty the plate by inverting plate and shaking contents out over the sink, keep inverted and tap dry on a thick layer of tissues.
   c. Add 200 µl of wash buffer to each well, wait 20 seconds, empty the plate as described in 5b.
   d. Repeat the washing procedure 5b/5c three times.
   e. Empty the plate and gently tap on thick layer of tissues.
6. Add 100 µl of diluted peroxidase-conjugate antibody to each well using the same pipetting order as applied in step 2. Do not touch the side or bottom of the wells.
7. Cover the tray and incubate the tray for 30 minutes at room temperature.
8. Repeat the wash procedure described in step 5a-e.
9. Add 100 µl of TMB substrate to each well, using the same pipetting order as applied in step 2. Do not touch the side or bottom of the wells.
10. Cover the tray and incubate the tray for 15 minutes at room temperature. It is advised to control the reaction on the plate regularly. In case of strong development the TMB reaction can be stopped sooner. Avoid exposing the microwell strips to direct sunlight. Covering the plate with aluminum foil is recommended.
11. Stop the reaction by adding 100 µl of stop solution with the same sequence and timing as used in step 9. Mix solutions in the wells thoroughly by gently swirling the plate. Gently tap the tray to eliminate any air bubbles trapped in the wells.
12. Read the plate within 30 minutes after addition of stop solution at 450 nm using a plate reader, following the instructions provided by the instrument's manufacturer.

*) In case plate washer is used, please note: use of a plate washer can result in higher background and decrease in sensitivity. We advise validation of the plate washer with the manual procedure. Make sure the plate washer is used as specified for the manual method.

10. INTERPRETATION OF RESULTS

- Calculate the mean absorbance for each set of duplicate standards, controls and samples.
- If individual absorbance values differ by more than 15% from the corresponding mean value, the result is considered suspect and the sample should be retested.
- The mean absorbance of the zero standard should be less than 0.3.
- Create a standard curve using computer software capable of generating a good curve fit. The mean absorbance for each standard concentration is plotted on the vertical (Y) axis versus the corresponding concentration on the horizontal (X) axis (logarithmic scale).
- If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.
- Samples that give a mean absorbance above the absorbance for the highest standard concentration are out of range of the assay. These samples should be retested at a higher dilution.

11. TECHNICAL HINTS

- User should be trained and familiar with ELISA assays and test procedure.
- If you are not familiar with the ELISA technique it is recommended to perform a pilot assay prior to evaluation of your samples. Perform the assay with a standard curve only following the instructions.
- Improper or insufficient washing at any stage of the procedure will result in either false positive or false negative results. Completely empty wells before dispensing wash buffer, fill with wash buffer as indicated for each cycle and do not allow wells to sit uncovered or dry for extended periods.
- Since exact conditions may vary from assay to assay, a standard curve must be established for every run. Samples should be referred to the standard curve prepared on the same plate.
- Do not mix reagents from different batches, or other reagents and strips. Remainders should not be mixed with contents of freshly opened vials.
- Each time the kit is used, fresh dilutions of standard, sample, detection antibody and buffers should be made.
- Caps and vials are not interchangeable. Caps should be replaced on the corresponding vials.
- To avoid cross-contaminations, change pipette tips between reagent additions of each standard, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- Waste disposal should be performed according to your laboratory regulations.

Technical support

For any questions or technical support related to the ELISA, please feel free to reach out to our technical support team at support@hycultbiotech.com. Hycult Biotech, Frontstraat 2a, 5405 PB Uden, the Netherlands

T: +31 (0)413 251 335, F: +31 (0)413 248 353

12. QUALITY CONTROL

The Certificate of Analysis included in this kit is lot-specific and is to be used to verify results obtained by your laboratory. The absorption values provided on the certificate of analysis are to be used as a guideline only. The results obtained by your laboratory may differ.

This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the immunoassay, the possibility of interference cannot be excluded.

For optimal performance of this kit, it is advised to work according to good laboratory practice.

13. PERFORMANCE CHARACTERISTICS

Precision and reproducibility

The inter-assay precision and reproducibility was determined with four plasma samples measured in duplicates over two independent experiments.

Plasma sample (n=9)	C3bBbP AU/ml (min-max)	CV%
Sample 1	108.2 (94 – 128)	10.3
Sample 2	320.1 (287 – 371)	8.8
Sample 3	55.7 (42 – 69)	14.0
Sample 4	73.4 (55 – 84)	11.8

Table 5

14. TROUBLESHOOTING

Warranty claims and complaints in respect of deficiencies must be logged before expiry date of the product. A written complaint containing lot number of the product and the generated data can be sent to support@hycultbiotech.com.

Suggestions summarized below in Table 6 can be used as guideline in case of unexpected assay results.

Low absorbance	High absorbance	Poor duplicates	All wells positive	All wells negative	Possible cause
•	•		•	•	Kit materials or reagents are contaminated or expired
•					Incorrect reagents used
•		•	•		Lyophilized reagents are not properly reconstituted
•	•	•	•	•	Incorrect dilutions or pipetting errors
•		•			Improper plastics used for preparation of standard and/or samples
•	•				Improper incubation times or temperature
		•			Especially in case of 37°C incubation: plates are not incubated uniformly
•					Assay performed before reagents had reached room temperature
•	•	•	•	•	Procedure not followed correctly
				•	Omission of a reagent or a step
		•			Poor mixing of samples
	•		•		Low purity of water
	•	•			Strips were kept dry for too long during/after washing
	•	•	•		Inefficient washing
	•	•			Cross-contamination from other samples or positive control
		•	•		TMB solution is not clear or colorless
•	•				Wrong filter in the microtiter reader
	•	•			Air bubbles
		•			Imprecise sealing of the plate after use
•					Wrong storage conditions
•					Lamp in microplate reader is not functioning optimally

Table 6