Rat Terminal Complement Complex (TCC)

HK106

Edition 11-19

ELISA KIT
PRODUCT INFORMATION & MANUAL

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



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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 rat terminal complement complex (TCC) ELISA kit is to be used for the *in vitro* quantitative determination of rat TCC 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 three distinct activation pathways of complement converge with the formation of a C5 convertase and it is cleavage of C5 by these convertases that initiates the lytic or terminal pathway. In contrast to the activation pathways, which require enzymatic cleavage for activation, the terminal pathway relies on conformational changes induced by binding. Binding of C6 facilitates binding of C7 which alters the conformation of the complex. After binding of C8 a variable number of C9 molecules associate with the C5b678 complex, the so called terminal complement complex (TCC) or Membrane Attack Complex (MAC). The formation of the membrane form of TCC causes lysis of cells or can trigger a variety of cellular metabolic pathways resulting in the synthesis and release of inflammatory mediators. The TCC contains neoantigens that are absent from the individual native components. Neoantigens are present both in the membrane-bound (MAC) and the fluid-phase (SC5b-9) complex. TCC is present in normal human plasma, < 1 AU/ml, and increases in patients with complement activation.

3. KIT FEATURES

- Working time of 3½ hours.
- Minimum concentration which can be measured is 0.31 mAU/ml.
- Measurable concentration range of 0.31 to 20 mAU/ml.
- Working volume of 100 µl/well.

Cross-reactivity

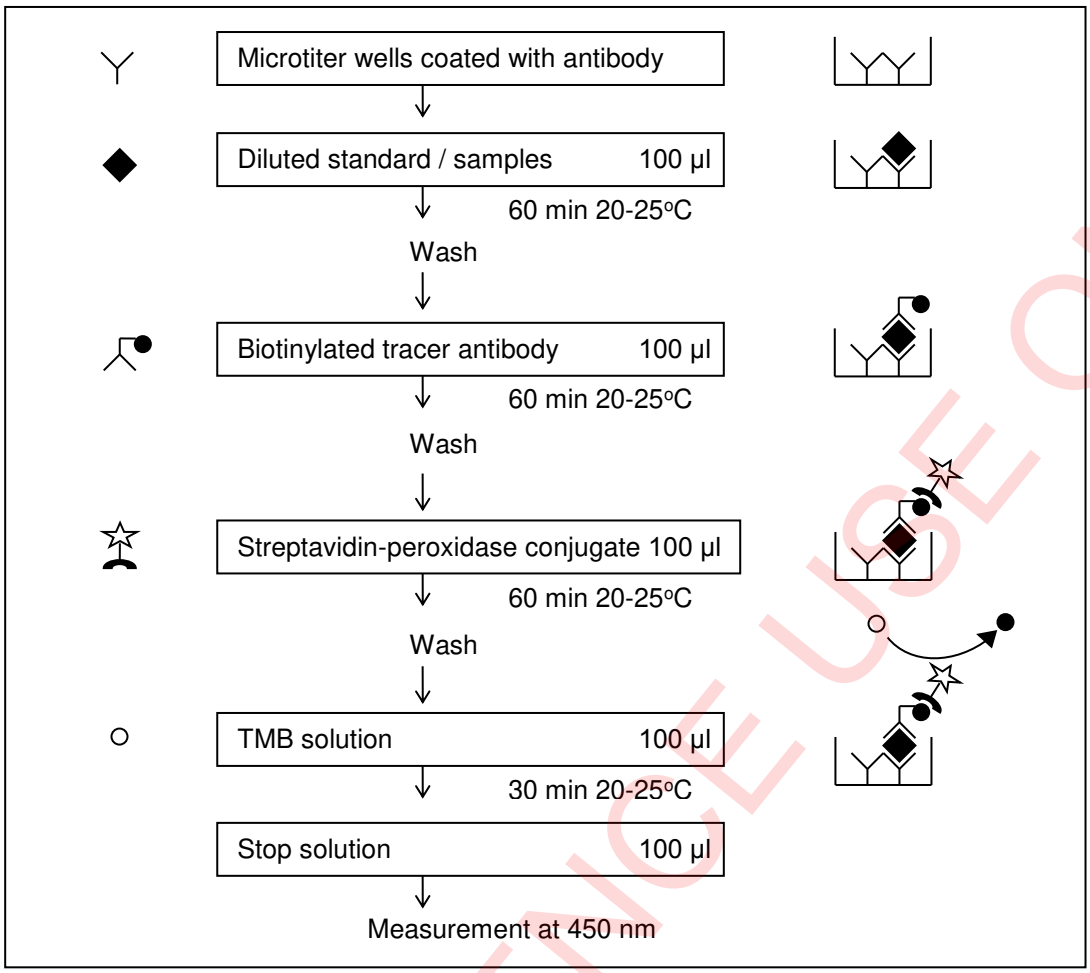
Potential cross-reacting proteins detected in the rat TCC ELISA:

Cross reactant	Reactivity
Human TCC	negative
Horse TCC	negative
Mouse TCC	negative
Pig TCC	negative
Rabbit TCC	negative

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

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4. PROTOCOL OVERVIEW



- The rat TCC ELISA is a ready-to-use solid-phase enzyme-linked immunosorbent assay based on the sandwich principle with a working time of 3½ hours.
- 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 rat TCC.
- Biotinylated tracer antibody will bind to captured rat TCC.
- Streptavidin-peroxidase conjugate will bind to the biotinylated tracer antibody.
- Streptavidin-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 rat TCC standards (log).
- The rat TCC concentration of samples, which are run concurrently with the standards, can be determined from the standard curve.

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5. KIT COMPONENTS AND STORAGE INSTRUCTIONS

Kit component	Cat.#	Quantity HK106-01	Quantity HK106-02	Color code
Wash buffer 20x	WB21	1 vial (60 ml)	1 vial (60 ml)	White
Dilution buffer 10x	DB97	1 vial (15 ml)	1 vial (15 ml)	Green
Standard		2 vials, lyophilized	4 vials, lyophilized	White
Tracer, biotinylated		1 vial, 1 ml lyophilized	2 vials, 1 ml lyophilized	White
Streptavidin-peroxidase 100x	CON04	1 tube, 0.25 ml in solution	1 tube, 0.25 ml in solution	Brown
TMB substrate	TMB050/TMB100	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		2	2	

Table 1

- 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 tracer in lyophilized form and streptavidin-peroxidase in solution are stable until the expiration date indicated on the kit label, if stored at 2 - 8°C.
- The exact amount of standard is indicated on the label of the vial and the Certificate of Analysis.
- Once reconstituted the standard is stable for 1 hour at room temperature. The standard cannot be stored for repeated use.
- Once reconstituted the tracer is stable for 1 month if stored at 2 - 8°C.
- The streptavidin-peroxidase can only be stored in concentrated solution and is not stable when stored diluted.
- 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 1 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.
- Adhesive covers can be ordered separately. Please contact your local distributor.
- Centrifuge for 1 ml tubes.

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6. WARNINGS AND PRECAUTIONS

- For research use only, not for diagnostic or therapeutic use.
- This kit should only be used by qualified laboratory staff.
- Do not under any circumstances add sodium azide as preservative to any of the components.
- Do not use kit components beyond the expiration date.
- Do not mix reagents from different kits and lots. The reagents have been standardized as a unit for a given lot. Use only the reagents supplied by manufacturer.
- The assay has been optimized for the indicated standard range. Do not change the standard range.
- Open vials carefully: vials are under vacuum.
- It is advised to spin down streptavidin-peroxidase tubes before use.
- 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.
- Incubation times, incubation temperature and pipetting volumes other than those specified may give erroneous 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.

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7. SAMPLE PREPARATION

Collection and handling

Serum or plasma

Rat TCC is measurable in all blood products, however, please notice correct collection of the samples is important to prevent the complement system from activating and obtaining false positive results.

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 1 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. Citrate and heparin do not block complement activation and are therefore not recommended.

Storage

Store samples below -20°C, preferably at -70°C in polypropylene tubes. Storage at -20°C can affect recovery of rat TCC. Use samples within 24 hours after thawing. Avoid multiple freeze-thaw cycles which may cause loss of rat TCC activity and give erroneous results.

Do not use hemolyzed, hyperlipemic, heat-treated or contaminated samples.

Dilution procedures

Before performing the assay, samples should be brought to 4°C and mixed gently. Prepare all samples (controls and test samples) prior to starting the assay procedure. Avoid foaming.

Plasma or serum samples

Rat TCC can be measured accurately if plasma samples are diluted at least 100x with supplied sample dilution buffer in polypropylene tubes.

Note that most reliable results are obtained with EDTA plasma. Citrate and heparin do not block complement activation and are therefore not recommended.

Remark regarding recommended sample dilution

The mentioned dilution for samples is a minimum dilution and should be used as a guideline. The recovery of rat TCC 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 rat TCC.

Do not use polystyrene tubes or sample plates for preparation or dilution of the samples.

Guideline for dilution of samples

Please see table 3 for recommended sample dilutions. Volumes are based on a total volume of at least 230 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.

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	Dilution	Pre-dilution	Amount of sample or pre-dilution required	Amount of Sample 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 3

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8. REAGENT PREPARATION

Allow all the reagents, with exception of the standard, samples and plates, to equilibrate to room temperature (20 – 25°C) prior to 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. Concentrated wash buffer may contain crystals. In case the crystals do not disappear at room temperature within 1 hour, concentrated wash buffer can be warmed up to 37°C. Do not shake the solution.

Dilution buffer

Prepare dilution buffer by mixing 15 ml of the 10x dilution buffer with 135 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.

Standard solution

The standard is reconstituted by pipetting the amount of dilution buffer mentioned on the CoA in the standard vial. Use the standard vial as Tube 1 in Figure 1. Once reconstituted the standard is stable for 1 hour at room temperature. Prepare each rat TCC standard in polypropylene tubes by serial dilution of the reconstituted standard with dilution buffer as shown in Figure 1*.

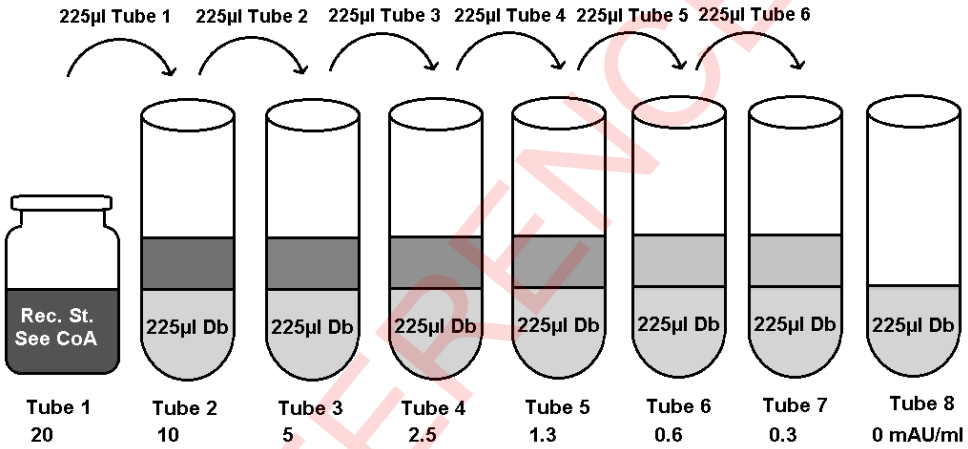


Figure 1

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

Tracer solution

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

Streptavidin-peroxidase solution

It is advised to spin down streptavidin-peroxidase tubes before use. Prepare the streptavidin-peroxidase solution by mixing 0.25 ml of the 100x streptavidin-peroxidase solution with 24.75 ml dilution buffer, which is sufficient for 2 x 96 tests. In case less volume is required, prepare the desired volume of streptavidin-peroxidase solution by diluting 1 part of the 100x streptavidin-peroxidase solution with 99 parts of dilution buffer.

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9. ELISA PROTOCOL

Bring all reagents to room temperature (20 - 25°C) before use. Samples should be brought to 4°C and mixed gently.

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 1 hour at room temperature.
5. Wash the plates 4 times with wash buffer 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 tracer 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 1 hour at room temperature.
8. Repeat the wash procedure described in step 5.
9. Add 100 µl of diluted streptavidin-peroxidase 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 1 hour at room temperature.
11. Repeat the wash procedure described in step 5.
12. 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.
13. Cover the tray and incubate the tray for 30 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 aluminium foil is recommended.
14. Stop the reaction by adding 100 µl of stop solution with the same sequence and timing as used in step 12. Mix solutions in the wells thoroughly by gently swirling the plate. Gently tap the tray to eliminate any air bubbles trapped in the wells.
15. 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.

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10. INTERPRETATION OF RESULTS

- Calculate the mean absorbance for each set of duplicate standards, control 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, tracer, streptavidin-peroxidase 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.
- The waste disposal should be performed according to your laboratory regulations.

Technical support

Do not hesitate to contact our technical support team at support@hycultbiotech.com for inquiries and technical support regarding the rat TCC ELISA.

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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 Hycult Biotech 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. 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 experimental data should sent to support@hycultbiotech.com.

Suggestions summarized below in Table 4 can be used as a guideline in the 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 were brought to 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
	•	•			Airbubbles
		•			Imprecise sealing of the plate after use
•					Wrong storage conditions
•					Lamp of microplate reader is not functioning optimally.

Table 4

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14. REFERENCES

1. Kotimaa, J et al; Functional assessment of rat complement pathway activities and quantification of soluble C5b-9 in an experimental model of renal ischemia/reperfusion injury. J Immunol Meth 2014, *412*:14

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