Tarcine BioMed (Hong Kong) Limited

Rta/IgG ELISA kit

PACKAGE INSERT

Consult Instructions for Use

(V03 /2013-10-12)

REF A-0148（48wells）A-0196（96wells） Σ 48 / 96

IVD 2-8℃

INTENDED USE

The NPCheck™ Rta/IgG ELISA Kit is for the qualitative detection of human IgG antibodies to Epstein-Barr viral Rta antigen in human serum by Enzyme-Linked Immunosorbent Assay (ELISA), to aid in diagnosis and clinical management of *the Nasopharyngeal carcinoma (NPC)*.

For *in vitro* diagnostic use only

INTRODUCTION

Detection of the Epstein-Barr virus (EBV) was first described in 1964 by Epstein, Achong, and Barr using electron microscopic studies of cultured lymphoblasts derived from patients with Burkitt's lymphoma[1]. EBV is classified as a member of the herpes-virus family based upon its morphology characteristics [2,3]. NPC is the most prevalent EBV-associated tumor, with a 100% detection rate of EBV genes in patients with undifferentiated NPC[4] subtypes. Recent studies demonstrated that an EBV immediate early gene, *BRLF1*, was frequently expressed in NPC tumors, and a significant elevation in immunoglobulin G (IgG) antibodies directed against *BRLF1* gene product Rta was detected in NPC sera[5,6]. The Rta/IgG is a blockbuster seriologic biomarker of the NPC[7-9] .

NPCheck™ Rta/IgG assay kit is developed with an *ELISA* method. In the multi-center clinical trials[10-14], NPC and control sera were examined with *Rta/IgG ELISA kit.* The results demonstrated that the Rta/IgG antibody titers are significantly higher in NPC. This ELISA test is sensitive and specific, and it may be useful as a diagnostic and screening parameter of NPC. The conclusive diagnosis of NPC is usually achieved with endoscopic guided biopsy and pathology study.

PRINCIPLE OF THE ASSAY

Enzyme-Linked Immunosorbent Assays (ELISA) rely on the ability of biological materials (i.e., antigens) to adsorb to plastic surfaces such as polystyrene (solid phase). When antigens bound to the solid phase are brought into contact with a patient's serum, antigen specific antibody, if present, will bind to the antigen on the solid phase forming antigen

antibody complexes. Excess antibody is removed by washing. This is followed by the addition of goat anti-human IgG conjugated with horseradish peroxidase which then binds to the antibody-antigen complexes. The excess conjugate is removed by washing, followed by the addition of Chromogen/Substrate, tetramethylbenzidine (TMB). If specific antibody to the antigen is present in the patient's serum, a blue color develops. When the enzymatic reaction is stopped with 2M H_2SO_4, the contents of the wells turn yellow. The color, which is indicative of the concentration of antibody in the serum, can be read on a suitable spectrophotometer or ELISA micro-well plate reader.

KIT PRESENTATION

Materials Supplied

Each kit contains the following components in sufficient quantities to perform the number of tests indicated on the package label.

1. **Rta antigen coated microplate: (48/96 wells)** configured in 4/8 breakapart 12-well snap-off strips; in a sealed aluminium foil pouch with desiccant. (48/96T: one plate).
2. **Sample Diluent:** 1 bottle containing 6/12 ml of buffer for sample dilution; coloured dark green; ready to use; green cap.
3. **Positive Control:** 1 bottle containing 1 ml Rta/IgG; coloured red; ready to use; red cap.
4. **Negative Control:** 1 bottle containing 1 ml buffer; coloured yellow; ready to use; green cap.
5. **Calibrator:** 1 bottle containing 1ml Rta/IgG positive buffer; coloured blue; ready to use; yellow cap.
6. **Horseradish-peroxidase (HRP) Conjugate:** 1 bottle containing 6/12 ml Goat anti-human IgG; coloured blue; ready to use; blue cap.
7. **Wash Buffer (20X concentrate):** 1 bottle containing 15/50 ml of a 20-fold concentrated buffer for washing wells; Coloureless solution; white cap.
8. **Chromogen/Substrate Solution A:** 1 bottle containing3/ 6 ml urea peroxide; Coloureless solution; ready to use; white cap. The reagent should remain closed when not in use.
9. **Chromogen/Substrate Solution B:** 1 bottle containing 3/6 ml Tetramethylbenzidine (TMB), Coloureless solution; ready to use; Black or brown cap. The reagent should remain closed when not in use.
10. **Stop Solution:** 1 bottle containing 3/6 ml 2M H_2SO_4 solution. Ready to use. yellow cap.
11. **Consult Instructions for Use**
12. **Quality Control Certificate**
13. **Re-sealable plastic bag:** 2 bags for storing the rest of strips of the coated plate.
14. **Cover membrane:** 1 pieces of adhesive plastic sheet for covering the plate during incubating procedure to prevent evaporation and potential pollution.

Materials and Equipment needed

1. Wash bottle, automated or semi-automated microplate washing system.
2. Distilled or deionized water (dH_20).
3. Disposable gloves and timer.
4. Graduated cylinders (25ml, 100ml, 1000ml).
5. Micropipettes, including single channel and multichannel, capable of accurately delivering 10-200 µL volumes.
6. Reagent reservoirs for multichannel pipettes.
7. Pipette tips .
8. Test tube for serum dilution .
9. Disposal basins and 0.5% sodium hypochlorite (50 mL bleach in 950 mL dH_20).
10. Thermostat incubator or water bath (37°C).
11. Microplate shaker and vortex tube mixer.
12. dual wavelength microplate reader, equipped for the measurement of absorbance at 450/630nm.

STORAGE AND STABILITY

If storage temperature is maintained between 2- 8° C, the reagents of the kit are stable up to 12 months. Refer to package label for manufacture date and expiration date. Care should be exercised to protect the reagents in this kit from contamination when the kit opened.

PRECAUTIONS

1. In compliance with article 1 paragraph 2b European directive 98/79/EC the use of the in vitro diagnostic medical devices is intended by the manufacturer to secure suitability, performances and safety of the product. Therefore the test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. The use of the test kits with analyzers and similar equipment has to be validated. Any change in design, composition and test procedure as well as for any use in combination with other products not approved by the manufacturer is not authorized, and the user himself is responsible for such changes. The manufacturer is not liable for false results and incidents for these reasons. The manufacturer is not liable for any results by visual analysis of the patient samples.
2. All reagents must be at room temperature (21° to 25° C) before running assay. Remove only the volume of reagents that is needed. Do not pour reagents back into vials as reagent contamination may occur.
3. The human serum components used in the preparation of the Controls in this kit have been tested for the presence of antibodies to human HBsAg, HCV as well as HIV 1&2 and found negative. Nevertheless, all materials should still be regarded and handled as potentially infectious.
4. Do not mix components from different production lots.
5. No reagents of other manufacturers should be used along with reagents of this test kit.
6. Do not use reagents after expiry date stated on the label.
7. Use only clean pipette tips, dispensers, and lab ware.
8. Do not interchange screw caps of reagent vials to avoid cross-contamination.
9. Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
10. After first opening and subsequent storage check conjugate and control vials for microbial contamination prior to further use.
11. To avoid cross-contamination and falsely elevated results pipette patient samples and dispense conjugate without splashing accurately to the bottom of wells.
12. Do not let wells dry during assay; add reagents immediately after completing wash steps.
13. Reagents may be irritating. Avoid contact with skin and eyes. In case of contact, flush with plenty of water.
14. If a sodium hypochlorite (bleach) solution is being used as a disinfectant, do not expose to work area during actual test procedure because of potential interference with enzyme activity.
15. Caution: Liquid waste at acid pH must be neutralized prior to adding sodium hypochlorite (bleach) solution to avoid formation of poisonous gas. Recommend disposing of reacted, stopped plates in biohazard bags.
16. Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.
17. The NPCheck™ Rta/IgG ELISA Kit is only designed for qualified personnel who are familiar with good laboratory practice.

WARNING: Avoid contact of Stop Solution (2M sulfuric acid) with skin or eyes. If contact occurs, immediately flush area thoroughly with plenty of water and consult a doctor!

SPECIMEN COLLECTION AND STORAGE

1. This assay was validated for use with human serum or plasma collected in sodium citrate, potassium EDTA, sodium EDTA, sodium heparin, lithium heparin. If separation is not sufficient for specimen preparation, specimens containing red blood cells or particulate matter may give inconsistent results.
2. Optimal performance of the kit depends upon the use of fresh serum samples: clear, non-hemolyzed, non-lipemic, and non-icteric.
3. Store serum or plasma between 2° and 8° C if testing will take place within 5 days after sample collection, otherwise they should be aliquoted and stored deep-frozen (-20 to -70°C).
4. Do not use a frost-free freezer because it may allow the specimens to go through freeze-thaw cycles and degrade antibody.
5. Samples that are improperly stored or are subjected to multiple freeze-thaw cycles may yield erroneous results. ***Avoid repeated freezing and thawing.***

METHODS FOR USE

Test Preparation

1. Please read the test protocol carefully **before** performing the assay. Result reliability depends on strict adherence to the test protocol as described.
2. The following test procedure is only validated for manual procedure. If performing the test on ELISA automatic systems, we recommend to increase the washing steps from three to five times and the volume of washing solution from 300µl to 350µl per well to avoid washing effects.
3. Perform all assay steps in the order given and without any appreciable delays between the steps.
4. A clean, disposable tip should be used for dispensing each control and sample.
5. Adjust the incubator to 37° ± 1°C.
6. All reagents must be removed from refrigeration and allowed to come to room temperature before use (21° to 25° C). Return all reagents to refrigerator promptly after use.
7. All samples and controls should be vortexed before use.
8. Dilute the 20X Wash Buffer with dH_20. Mix well.
9. Prior to commencing the assay, the distribution and identification plan for all specimens and controls should be carefully established on the result sheet. Select the required number of microtiter strips or wells and insert them into the holder. Please allocate at least:

    1 well (e.g. A1) for substrate blank

    1 well (e.g. B1) for negative control

    1 well (e.g. C1) for positive control

    2 wells (e.g. D1+E1) for the Calibrator

Assay Procedure

1. Dispense 100µl Sample Diluent into sample wells, then dispense 10µl sample and 100µl controls and Calibrators into their respective wells. Shake well, and ensure the samples are properly mixed.
2. Cover wells with the cover membrane supplied in the kit.
3. **Incubate for 30min at 37±1°C.**
4. When incubation has been completed, remove the cover membrane, aspirate the content of the wells and wash each well with 300µl of Washing Solution. Avoid overflows from the reaction wells. Aspirate or shake out to remove all liquid. Repeat the wash procedure for manual or semi-automated equipment or four times (for a total of five washes) for automated equipment. The soak time between each wash cycle should be more than 5 sec. At the end, carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!

Note: Washing is critical! Insufficient washing results in poor precision and falsely elevated

5. Dispense 100µl Conjugate into all wells Cover with cover membrane supplied in the kit.
6. **Incubate for 20min at at 37±1°C.** *Do not expose to direct sunlight.*
7. Repeat step 4.
8. Dispense 50µl Chromogen/Substrate Solution A and 50µl Chromogen/Substrate Solution B into all wells
9. **Incubate for exactly 10 min at 37±1°C in the dark.**
10. Dispense 50 µl Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution.

    Any blue colour developed during the incubation turns into yellow.

11. Measure the absorbance (A value) of the specimen at 450/630nm within 30 min after addition of the Stop Solution. Where applicable calculate the **mean A values** of all duplicates.

Measurement

1. Adjust the ELISA Microplate Reader **to zero** using the **substrate blank in well A1**.
2. Cut off value (C.O.) calculation:

    C.O. = **mean A value of Calibrator**

3. Validation Criteria

In order for an assay to be considered valid, the criteria in the **Quality Control Certificate for each Lot.** must be met**.** If these criteria are **NOT** met, the test is **NOT VALID** and must be repeated.

If above criteria are not met upon repeat testing, contact Tarcine Technical Services.

INTERPRETATION OF RESULTS

1. Samples are considered **POSITIVE** if the A value is higher than 10% over the cut-off.
2. Samples are considered **NEGATIVE** if the A value is lower than 10% below the cut-off.
3. **Grey zone:** Samples with an A value of 10% above or below the cut-off should not be considered as clearly positive or negative but in a grey zone. It is recommended to repeat the test again 2 - 4 weeks later with a fresh sample. If results in the second test are again in the grey zone the sample is to be considered **NEGATIVE**.
4. In a multi-center clinical trial, 81% (384/473) of NPC samples had positive Rta/IgG result; 92.2% (502/545) of normal control samples had negative Rta/IgG result. Therefore, it has been proposed that serum/plasma Rta/IgG analysis may be incorporated usefully as a diagnostic and screening parameter of NPC. The noninvasive and cost-effectiveness of screening can be enhanced further by targeting individuals who have an even greater risk of NPC, such as middle-aged men and those with a family history of NPC.
5. Further studies will be needed to address whether individuals who have persistently positive Rta/IgG results have NPC, such as nasopharyngeal tissue biopsy and pathology study, etc. A precise diagnosis should take into consideration clinical history, symptomatology as well as serological data.

LIMITATIONS OF USE

1. The user of this kit is advised to carefully read and understand the package insert. Strict adherence to the protocol is necessary to obtain reliable test results. In particular, correct sample and reagent pipetting, along with careful washing and timing of the incubation steps is essential for accurate results.
2. The values obtained from this assay are intended to be an aid to diagnosis of NPC. A single result is not reliable for diagnosis, and periodically repeated assays are necessary. Each physician must interpret the results in light of the patient's history, physical findings and other diagnostic procedures.
3. All immunology experiment system with high sensitivity has potential nonspecific. Therefore, any unacceptable positive results may be caused by biological false positive of ELISA method.
4. There is a possibility of assay cross-reactivity with specimens containing anti-*E.coli* antibody.
5. The performance characteristics for this assay have not been established for pediatric specimens.
6. Results obtained from immunocompromised individuals should be interpreted with caution.

PERFORMANCE CHARACTERISTICS

Precision

1. Studies were performed to document typical assay precision with the NPCheck™ Rta/IgG ELISA Kit. The mean, SD, and CV % were calculated for Intra-and Inter-Assay Precision.
2. CV (Coefficients of Variation) was determined using data from a Rta/IgG positive sera. The intra-assay CVs are based on 10 measurements for each serum on three lots and the inter-assay CVs on 10 well measurements per plate repeated on three different lots.

Inter-assay	n	O.D. mean	SD	CV%
Pos. Serum	3 lots X 10 Tests	0.661	0.03	**5.3**

Intra-assay	n	O.D. mean	SD	CV%
Pos. Serum	**10 wells X 3 lots**	**0.546**	**0.05**	**9.2**

- **Diagnostic Specificity: 100%**

The diagnostic specificity is defined as the probability of the assay of scoring negative in the absence of the Rta/IgG. 20 Rta-negative controls were tested with three lots of NPCheck™ Rta/IgG ELISA Kit products.

- **Diagnostic Sensitivity: 100%**

The diagnostic sensitivity is defined as the probability of the assay of scoring positive in the presence of the

Rta-IgG. 10 Rta-positive controls were tested with three lots of NPCheck™ Rta/IgG ELISA Kit products.

Interferences

1. Interferences with hemolytic, lipemic or icteric sera are not observed up to a concentration of 24 g/dL hemoglobin, 62 mg/dL triglycerides and 50.8µmol/L bilirubin.
2. Negative test results for all 100 samples indicate an absence of cross-reactivity with ANA, RF, and ENA.

Note: *The results refer to the groups of samples investigated; these are not guaranteed specifications.*

REFERENCES

1. Epstein, M.A., B.B. Achong, and Y.M. Barr. 1964. *Lancet.* 1: 702-703.

2. Epstein, M.A., Y.M. Barr, and B.G. Achong. 1965. *Wistar Inst. Sympos. Monogr.* 4: 69-82.

3. Schooley, R.T. and R. Dolin. 1985. Epstein-Barr Virus (Infectious Mononucleosis). In: *Priciples and Practice of Infectious Diseases*, 2nd Edition. Mandell, G. L., R. G. Douglas, and J. E. Bennett. (eds). John Wiley and Sons, New York. pp 97 - 982.

4. Rickinson A.B, Kieff E. 1996. Epstein–Barr virus. In: Field's Virology, 3rd edn. Fields B.N, Knipe D.M, Howley P.M. (eds). Lippincott-Raven, Philadelphia. pp 2397–2446.

5. Feng, P., E.C. Ren, D. Liu, et al. 2000. *Journal of General Virology*, 81: 2417-2423.

6. Feng, P., S.H. Chan, M.R. Soo, et al. 2001. *Cancer,* 92:1872-1880.

7. Zalani, S., E. Holley-Guthrie, S. Kenney. 1996. *Proc. Natl. Acad. Sci. USA*. 93:9194-9199

8. Kieff, E. 1996. Epstein-Barr Virus and its replication. In: Field's Virology, 3rd edn. Fields B.N, Knipe D.M, Howley P.M. (eds). Lippincott-Raven, Philadelphia. pp 2342–2396.

9. Klein E, L.L. Kis, G. Klein, 2007. *Oncogene,* 26: 1297-1305.

10. Ren, J., X.M. Zhang, X.G. Zhang, et al. 2006. *Chin J. Microbiol. Immunol.,* 26:1057-1059.

11. Zheng, Y.M., Y.L. Cai, J.R. Cheng, et al. 2009. *Chin. J. Exp. Clin. Virol.,* 23:285-287.

12. Zhu, W.L., X.Q. Liang, Y. Zhang, et al. 2009. *Chin J. of Oncol. Prev. and Treat.*, 1:211-213.

13. Guo, L.P., Y. Cui, W.Z. Li, et al. 2012. *Guide of China Medicine,* 10:26-27

14. Li, X.L., Y. Chen, R.Q. Zheng, et al. 2013. *Laboratory Medicine and Clinic,* 10:321-323

EXPLANATION OF SYMBOLS

Temperature limitation

IVD — In Vitro Diagnostic Medical Device

REF — Catalogue number

Σ — Contains sufficient for tests

LOT — Batch code

CAL — Calibrator

 Date of manufacture

 Manufacturer

 Consult Instructions for Use

EC REP — Authorized Representative

CONT — Contents of kit

ORDERING INFORMATION

REF Rta/IgG ELISA Kit 96 wells A-0196 48 wells A-0148

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