Creative Diagnostics®

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User's Manual

Anti-polyomavirus BK (BKV) IgG ELISA Kit

REF **DEIASL161**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

The kit is intended for professional use for the qualitative and semi-quantitative detection of species-specific IgG antibodies against polyomavirus BK (BKV) in human serum and plasma. **For research use only, not for use in diagnostic procedures.**

Principles of Testing

Anti-polyomavirus BK (BKV) IgG ELISA Kit is a solid-phase immunoanalytical test. The strips are coated with recombinant species-specific BKV antigens. If antibodies are present in the test samples, they will bind to the immobilized proteins. The bound antibodies then react in the next step with horseradish peroxidase-labeled anti-human IgG antibodies. The amount of bound labeled antibodies is determined by a color enzymatic reaction. Negative samples do not react, a slight change in the color of the wells is the background of the reaction.

Reagents And Materials Provided

1. ELISA break-away strips in the handling frame coated with the specific antigens, 8×12 wells
2. 1.3 mL Negative control human serum, ready to use, 1 vial
3. 1.3 mL Calibrator (human serum), ready to use, 1 vial
4. 1.3 mL Positive control human serum, ready to use, 1 vial
5. 13 mL Anti-human IgG animal antibodies labelled with horseradish peroxidase (anti-IgG Px conjugate), ready to use, 1 vial
6. 55 mL Wash buffer, 10× concentrated, 1 vial
7. 60 mL Dilution buffer, ready to use, 1 vial
8. 13 mL Chromogenic substrate TMB-O (TMB/H_2O_2), ready to use, 1 vial
9. 13 mL Stop solution (0.4 M sulfuric acid) , ready to use, 1 vial

   Notice: Control sera may be colorless to yellowish or blue due to the use of different diluents.

Materials Required But Not Supplied

Distilled/deionised water for dilution of the Wash buffer 10× , pipetting equipment, equipment for liquid dispensing and strip washing, spectrophotometer/colorimeter. All instruments and devices used must have a valid function validation.

Storage

The ELISA kit should be used within three months after opening.

1. Store the kit and the kit reagents at +2 to +10°C, in a dry place and protected from the light. Under these conditions, the expiration date is indicated at the ELISA kit label and at all reagent labels.

2. Kits are shipped in cooling bags, the transport time of 72 hours have no influence on expiration. If you find damage at any part of the kit, please inform the manufacturer immediately.
3. Store unused strips in the sealable pouch and keep the desiccant inside.
4. Store undiluted serum samples at +2 to +10°C up to one week. For longer period make aliquots and keep them at -18 to -28°C. Avoid repeated thawing and freezing.
5. Solutions of diluted serum samples at working concentration cannot be stored. They need to be prepared fresh.

Reagent Preparation

1. Allow all kit components to reach room temperature.
2. Thoroughly mix Dilution buffer, Conjugate anti-IgG Px and Chromogenic substrate.
3. Thoroughly mix tested samples and control sera just prior to testing. Tested samples dilute 101× with Dilution buffer (eg 5 µL sample + 500 µL Dilution buffer). Do not dilute control sera and calibrator, they are in working concentration (ready to use).
4. Prepare a working concentration of Wash buffer 10× by diluting it 10× in a suitable volume of distilled/deionized water (eg. 50 mL of Wash buffer 10× + 450 mL H_2O). If there are salt crystals in the concentrated solution, warm it in a water bath of + 32°C to + 37°C and mix well before diluting. Unused wash solution in working concentration can be stored for 1 month at room temperature.
5. Do not dilute Conjugate anti-IgG Px, Chromogenic substrate and Stop solution, they are ready to use.

Assay Procedure

The manufacturer is not responsible for the correct function of the kit if the assay procedure is not followed.

1. Allow strips, vacuum sealed with desiccant, to reach room temperature before opening the bag, to avoid dew condensation of the plate. Prepare the required number of strips for the reaction. Seal unused strips together with the desiccant in a zipper bag or seal under vacuum.
2. Fill the wells with 100 µL of Standards and diluted samples according to the pipetting scheme (Figure 1). Start with filling the first well with Negative control serum. Then fill the next two wells with Calibrator and next well with Positive control serum. Fill the remaining wells with diluted samples (S1, S2, S3, ...). It is sufficient to apply samples as singlets, however, if you wish to minimize the laboratory error apply Calibrator in triplet and the samples and control sera in doublets. We recommend to include positive reference serum sample (your in-house internal control) into each run to follow the sequence, variability and accuracy of calibration. Incubate 30 minutes (+/- 2 min) at room temperature.
3. Aspirate the contents of the wells into a safety collection bottle containing a suitable disinfectant. Then wash the wells 4 times with 250 µL of wash solution. Avoid overflowing the solution out of the wells. Aspirate the contents of the wells and tap the plate on an adsorbent paper.
4. Mix thoroughly the vial of anti-IgG Px conjugate and pipette 100 µL of anti-IgG Px conjugate into the wells. Incubate 30 minutes (+/- 2 min) at room temperature.
5. Aspirate the fluid from the wells and wash them with 4 x 250 µL of wash solution. Aspirate and tap.
6. Pipette 100 µL of Chromogenic substrate solution into the wells. Incubate for 10 minutes (+/- 30 sec) in the dark at room temperature. Start measuring the incubation time after pipetting the first strip of the plate. Follow this rule to avoid breaking the time interval. Pipette quickly at regular rhythm, or use a suitable

dispenser. Cover the strips with foil, an opaque lid, or keep them in a dark place for the duration of the reaction.

7. Stop the reaction by adding 100 µL of Stop solution. Pipette at the same rate as the Chromogenic substrate so that the enzymatic reaction proceeds in all wells at the same time. Check that there are no bubbles in the wells, if so, gently tap the plate frame to remove them.

9. Measure the intensity of the colour reaction on a spectrophotometer/colorimeter at 450 nm within 10 minutes after stopping the reaction. We recommend using a 620-690 nm reference filter.

Figure 1: Scheme of application of samples

	1	2	3	4	5	6	7	8	9	10	11	12
a	NC											
b	CAL											
c	CAL											
d	PC											
e	S1											
f	S2											
g	S3											
h	S...											

Interpretation Of Results

50-80% of the population is infected with Polyoma BK virus in childhood. Infection occurs without symptoms and passes into a latent stage, which is associated with long-term presence of anamnestic IgG antibodies in the serum. BKV occurs in four genotypes, characterized by a different sequence of major neutralizing antigenic determinants on the major capsid protein VP1. Representation of individual genotypes in the general population is regionally different: genotype I prevails worldwide (47-82%), followed by genotype IV (5-54%). Genotypes II - III are rarer (0-9%). In latently infected people, the virus may be reactivated repeatedly or they may be reinfected with another virus strain. Reactivation/reinfection can be associated with transient viremia or asymptomatic viral shedding in urine. In immunodeficient patients, reactivation or reinfection may cause diseases of the urogenital tract, in rare cases even a generalized infection associated with various types of organ disorder. A high risk of complications is associated primarily with primary infection of the patient in a state of immunosuppression, so it is appropriate to know the serostatus of the graft donor in patients with transplant, particularly in recipients of the kidney transplant. The recombinant antigens used in the assay include two of the most frequently represented genotypes of BKV (type I and IV) and do not cross-react with other human polyomaviruses (polyoma JC, Merkel cell polyomavirus). However, it can cross-react with monkey polyomavirus SV40.

Evaluation

1. Qualitative orientation evaluation

a. Calculate the mean OD value of the Calibrator from the two wells. If you are applying three Calibrator wells and some of these values differ by more than 20% from the mean, do not use it for calculation and calculate the mean of the remaining two values.

b. Determine the cut-off value by multiplying the mean OD value of the Calibrator by the correction factor.

The value of the correction factor is stated in the Quality Control Certificate for the given kit lot.

c. Samples with an OD value < 90 % cut-off are negative and samples with an OD value > 110 % cut-off are considered positive.

2. Semiquantitative evaluation

Determine Positivity Index for each sample:

a. First determine the cut-off value as in the previous evaluation method.

b. Determine the index value for each sample by dividing the OD of the test sample by the cut-off value.

c. Read the appropriate degree of reactivity of the sample.

RESULTS EVALUATION

Positivity index	Evaluation
< 0.90	Negative
0.90 – 1.10	+/-
> 1.10	Positive*

*** on the basis of the Positivity Index value it is possible to estimate semiquantitatively the amount of antibodies in the sample**

Example

Obtained OD Calibrator = 0.814; 0.876

Mean OD Calibrator = 0.845

OD sample = 0.800

Correction factor Calibrator = 0.37

Cut-off value = 0.845 × 0.37 = 0.313

Positivity index value = 0.800 / 0.313 = 2.56

Note: A rating of +/- means that the sample is in the gray zone. Repeat the test for this result. If the sample is again in the gray zone after retesting, repeat the test with an alternative method or use a sample from a new sample from the same individual 1-2 weeks later.

Precision

The interassay variability (between tests) and the intraassay variability (within the test) were determined by testing samples with different OD values.

The variation coefficient of intraassay is max. 8 %. It is measured for each particular lot at least on 12 parallels of the same microtiter plate.

Example: (n = number of parallel wells on the same plate)

n	A	±σ	CV rep.
16	1.893	0.032	2.6 %

The variation coefficient of reproducibility is a maximum of 15 %. It is measured for each lot by comparing the wells of the same sample in several consecutive tests.

Example: (n = number of tests of a certain sample)

n	A	±σ	CVrepro
6	0.341	0.043	12.6%
6	1.525	0.128	8.4 %
6	2.24	0.088	3.9%

Detection Range

The measuring range is determined by the measuring capability of the spectrophotometer / colorimeter used.

Sensitivity

The analytical sensitivity of the assay is defined as the mean of the sample without analyte plus three times of the standard deviation and represents the lowest detectable antibody titer. The analytical sensitivity value is determined for each kit lot and is stated in the Certificate of Analysis of that kit lot.

Specificity

The quality of the specific recombinant BKV virus antigens used, which recognize specific antibodies in patient samples, ensure the high specificity and sensitivity of this assay. However, there is some degree of crossreactivity of antibodies against other infectious agents

Recovery

Measured values of recovery test for every Lot are between 80-120 % of expected value.

Interferences

Haemolytic and lipemic samples have no influence on the test results up to concentration of 50 mg/mL of haemoglobin, 5 mg/mL of bilirubin and 50 mg/mL of triglycerides. Nevertheless, such samples can only be tested with reservations.

Precautions

1. All kit components are for research use only.
2. The manufacturer guarantees the usability of the kit as a whole.
3. Wash buffer, Chromogenic substrate, Stop solution, and Dilution buffer are inter-changeable between CD kits, unless otherwise noted in the kit instructions.
4. Work aseptically to avoid microbial contamination of samples and reagents.
5. When collecting, diluting, and storing reagents, be careful not to cross-contaminate them or contaminate them with enzymatic activity inhibitors.
6. The Chromogenic Substrate shouldn' t come into contact with oxidizing agents and metal surfaces. Because it is sensitive to light, close the bottle immediately after use. The Chromogenic substrate must be clear in

use. Do not use the solution if it is blue.

7. Follow the Instruction manual exactly. Non-reproducible results may arise in particular:
   a. insufficient mixing of reagents and samples before use
   b. inaccurate pipetting and non-compliance with the incubation times given in Assay Procedure
   c. poor washing technique and splashing of the edges of the wells with sample or conjugate
   d. using the same tip when pipetting different solutions or swapping caps
8. Human control sera and standards used in the kit were tested for the absence of HBsAg, HCV and anti-HIV-1,2 antibodies. Treat test specimens, control sera, standards, and used strips as infectious material. Autoclave items that have been in contact with them for 1 hour at 121°C or disinfect for at least 30 minutes with 3% chloramine solution.
9. Neutralize liquid waste containing Stop solution (sulfuric acid solution) with 4% sodium bicarbonate solution before disposal.
10. Disinfect the waste generated during strip washing in a waste container using a suitable disinfectant solution (eg Incidur, Incidin, chloramine, ...) at the concentration recommended by the manufacturer.
11. Handle Stop solution carefully to avoid splashing on the skin or mucous membranes. If this happens, wash the affected area with plenty of running water.
12. Do not eat, drink or smoke while working. Do not pipette by mouth, but by suitable pipetting devices. Wear protective gloves and wash your hands thoroughly after work. Be careful not to spill specimens or form an aerosol.
13. All reagents and packaging material must be disposed of in accordance with applicable legislation.
14. In case of suspicion of an adverse event in connection with the use of the kit, inform the manufacturer and the competent state authority without delay.