



User's Manual

Anti-polyomavirus BK (BKV) IgG ELISA Kit



DEIASL161



96T



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

ELISA anti-BKV IgG 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 STRIPS Ag 1 × 12 pcs
2. 1.3 mL Negative control human serum, r.t.u. NC 1 vial
3. 2.0 mL Calibrator (human serum), r.t.u. CAL 1 vial
4. 1.3 mL Positive control human serum, r.t.u. PC 1 vial
5. 13 mL Anti-human IgG animal antibodies labelled with horseradish peroxidase (anti-IgG Px conjugate) r.t.u. CONJ 1 vial
6. 55 mL Wash buffer, 10x concentrated WASH 10x 1 vial
7. 60 mL Dilution buffer, r.t.u. DIL 1 vial
8. 13 mL Chromogenic substrate TMB-O, r.t.u. (TMB/H₂O₂) TMB-O 1 vial
9. 13 mL Stop solution, r.t.u. (0.4 M sulfuric acid) STOP 1 vial
10. Instruction manual
11. Quality Control Certificate

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 WASH 10x, pipetting equipment, equipment for liquid dispensing and strip washing, spectrophotometer/colorimeter.

All instruments and devices used must have a valid function validation.

Storage

2°C - 10°C



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Reagent Preparation

1. Allow all kit components to reach room temperature.
2. Thoroughly mix Dilution buffer DIL, Conjugate anti-IgG Px CONJ and Chromogenic substrate TMB-O.
3. Thoroughly mix tested samples and control sera just prior to testing. Tested samples dilute 101 \times with Dilution buffer DIL (eg 5 μ L sample + 500 μ L Dilution buffer). Do not dilute control sera and calibrator, they are in working concentration (r.t.u., ready to use).
4. Prepare a working concentration of Wash buffer WASH 10 \times by diluting it 10 \times in a suitable volume of distilled/deionized water (eg. 50 mL of WASH 10 \times + 450 mL H₂O). 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 CONJ, Chromogenic substrate TMB-O and Stop solution STOP, 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 STRIPS Ag, 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 μ L of Standards and diluted samples according to the pipetting scheme (Figure 1). Start with filling the first well with Negative control serum NC. Then fill the next two wells with Calibrator CAL and next well with Positive control serum PC. 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 CAL 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 (see Precautions). Then wash the wells 4 times with 250 μ L 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 CONJ and pipette 100 μ L of anti-IgG Px conjugate CONJ 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 μ L of wash solution. Aspirate and tap.
6. Pipette 100 μ L of Chromogenic substrate TMB-O 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 μ L of Stop solution STOP. Pipette at the same rate as the Chromogenic substrate TMB-O 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.
8. 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...											

Calculation

1. Qualitative orientation evaluation

- Calculate the mean OD value of the Calibrator CAL from the two wells. If you are applying three Calibrator CAL 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.
- Determine the cut-off value by multiplying the mean OD value of the Calibrator CAL by the correction factor. The value of the correction factor is stated in the Quality Control Certificate for the given kit lot.
- 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:

- First determine the cut-off value as in the previous evaluation method (See paragraph 8.1, point 2).
- Determine the index value for each sample by dividing the OD of the test sample by the cut-off value.
- Read the appropriate degree of reactivity of the sample (See RESULTS EVALUATION).

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 CAL = 0.814; 0.876

Mean OD Calibrator CAL = 0.845

OD sample = 0.800

Correction factor Calibrator CAL = 0.37

Cut-off value = 0.845 x 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.

Interpretation Of Results

1. 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.

Performance Characteristics

1. Validity of the test

The OD values of the standards / control sera and the ratio of the OD values of the standards PC / CAL should be within the ranges stated in the Quality Control Certificate of the lot.

The Calibrator and Controls are human sera, and as such they may show inhomogeneity, if their value in the test is significantly different from the values stated in the Certificate of analysis (see CoA - lot characteristics), consult the results with the manufacturer.

Precision

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

1. Repeatability (intraassay)

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	$\pm\sigma$	CV rep.
16	1.893	0.032	2.6 %

2. Reproducibility (interassay)

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	$\pm\sigma$	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 Quality Control Certificate 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 laboratory use only.
2. The manufacturer guarantees the usability of the kit as a whole.
3. Wash buffer WASH 10x, Chromogenic substrate TMB-O, Stop solution STOP, and Dilution buffer DIL are interchangeable between ELISA 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 TMB-O 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 TMB-O 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:
insufficient mixing of reagents and samples before use
inaccurate pipetting and non-compliance with the incubation times given in ASSAY PROCEDURE
poor washing technique and splashing of the edges of the wells with sample or conjugate
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 antiHIV-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.
10. Handle Stop solution STOP carefully to avoid splashing on the skin or mucous membranes. If this happens, wash the affected area with plenty of running water.
11. 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.
12. All reagents and packaging material must be disposed of in accordance with applicable legislation.
13. 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.

