

ELISA-VIDITEST

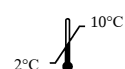
anti-BKV IgG

REF

ODZ-405



96 tests



2°C - 10°C



Distribuito in ITALIA da

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Type of determination: IgG antibodies

Type of evaluation: Qualitative, Semiquantitative

Type of samples: Serum/Plasma

Processing possibility: Manual



Instruction manual

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1. TITLE

ELISA-VIDITEST anti-BKV IgG

2. INTENDED USE

The kit is intended for professional use for the qualitative and semiquantitative detection of species-specific IgG antibodies against polyomavirus BK (BKV) in human serum and plasma for diagnostics of diseases caused by or associated with BKV infection (e.g. polyomavirus nephropathy, hemorrhagic cystitis, urethral stenosis), for differential diagnosis of generalized infections in immunodeficient patients and to determine the risk of these complications associated with immunosuppressive therapy.

3. TEST PRINCIPLE

ELISA-VIDITEST 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.

4. KIT COMPONENTS

ELISA break-away strips in the handling frame coated with the specific antigens	STRIPS Ag	1 x 12 pcs
1.3 mL Negative control human serum, r.t.u. ¹⁾	NC	1 vial
2.0 mL Calibrator (human serum), r.t.u.	CAL	1 vial
1.3 mL Positive control human serum, r.t.u.	PC	1 vial
13 mL Anti-human IgG animal antibodies labelled with horseradish peroxidase (anti-IgG Px conjugate) r.t.u.	CONJ	1 vial
55 mL Wash buffer, 10x concentrated	WASH 10x	1 vial
60 mL Dilution buffer, r.t.u.	DIL	1 vial
13 mL Chromogenic substrate TMB-O, r.t.u. (TMB/H ₂ O ₂)	TMB-O	1 vial
13 mL Stop solution, r.t.u. (0.4 M sulfuric acid)	STOP	1 vial
Instruction manual		
Quality Control Certificate		
¹⁾ r.t.u., ready to use		

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

Chromogenic substrate TMB-O is compatible and interchangeable between ELISA-VIDITEST kits which contain TMB-O and not compatible with other Chromogenic substrates used in other ELISA-VIDITEST TMB, TMB-BF.

5. MATERIALS REQUIRED BUT NOT PROVIDED

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.

6. REAGENTS PREPARATION

- Allow all kit components to reach room temperature.
- Thoroughly mix Dilution buffer [DIL], Conjugate anti-IgG Px [CONJ] and Chromogenic substrate [TMB-O].
- Thoroughly mix tested samples and control sera just prior to testing. Tested samples dilute 101x with Dilution buffer [DIL] (eg 5 µL sample + 500 µL Dilution buffer). Do not dilute control sera and calibrator, they are in working concentration (r.t.u., ready to use).
- Prepare a working concentration of Wash buffer [WASH] [10x] by diluting it 10x in a suitable volume of distilled/deionized water (eg. 50 mL of [WASH] [10x] + 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.
- Do not dilute Conjugate anti-IgG Px [CONJ], Chromogenic substrate [TMB-O] and Stop solution [STOP], they are ready to use.

7. ASSAY PROCEDURE

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

- 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.
- 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 [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**.

- Aspirate the contents of the wells into a safety collection bottle containing a suitable disinfectant (see WARNINGS). 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.
- Mix thoroughly the vial of anti-IgG Px conjugate [CONJ] and pipette 100 µL of anti-IgG Px conjugate [CONJ] into the wells.

Incubate **30 minutes** (+/- 2 min) **at room temperature**.

- Aspirate the fluid from the wells and wash them with 4 x 250 µL of wash solution. Aspirate and tap.
- Pipette 100 µL 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.

- g. Stop the reaction by adding 100 µL 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.
- h. 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...											

8. TEST EVALUATION

8.1 Qualitative orientation evaluation

1. 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.
2. **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.**
3. Samples with an OD value < 90 % cut-off are negative and samples with an OD value > 110 % cut-off are considered positive.

8.2 Semiquantitative evaluation

Determine Positivity **Index** for each sample:

1. First determine the cut-off value as in the previous evaluation method (See paragraph 8.1, point 2).
2. Determine the index value for each sample by dividing the OD of the test sample by the cut-off value.
3. 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.

9. RESULT INTERPRETATION

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.

10. TEST CHARACTERISTICS

The kit is intended for the qualitative and semiquantitative detection of anti-BKV IgG antibodies in human serum and plasma. Suitable specimens are serum and plasma (heparinised) samples obtained by standard laboratory techniques.

10.1 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.

10.2 Precision of the test

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

10.2.1 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 %

10.2.2 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%

10.2.3 Recovery test

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

10.3 Diagnostic sensitivity and specificity of the test

Since there is no commercially available kit for the determination of type-specific antibodies against BKV, a test of the diagnostic efficiency was performed using serum or plasma of defined donors (recipients of kidney transplant, blood donors, children aged 1 year) and on the basis of comparative testing of a panel of serum delivered by the National Reference Laboratory for Papillomaviruses and Polyomaviruses. In total, 222 serum or plasma samples were examined. The prevalence of antibodies found in each serum/plasma group was consistent with the assumption and with the published data: a high antibody prevalence (83 and 87%) was detected in the plasma group from the Tx kidney recipients and in the serum group from healthy blood donors, similar to seroprevalence studies by Keana et al., 2009, or Stolt et al., 2003. IgG antibodies against BKV were detected in all 10 Tx kidney recipients and were detected by PCR for active BKV infection. In children aged 1 year, who had previously not been infected with BKV and had already decreased maternal antibody levels, seroprevalence of 5% was found. In a comparative test of 50 samples that were tested by the reference method in the NRL, a 93% agreement was found.

These results show that ELISA-VIDITEST ant-BKV IgG has sufficient diagnostic sensitivity and specificity.

10.4 Analytical sensitivity of the test

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.

10.5 Analytical specificity of the test

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 cross-reactivity of antibodies against other infectious agents, see Chapter 9 (RESULT INTERPRETATION).

10.6 Measuring range

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

10.7 Interference

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.

11. WARNINGS

- a. All kit components are for laboratory use only.
- b. The manufacturer guarantees the usability of the kit as a whole.
- c. Wash buffer [WASH] [10x], Chromogenic substrate [TMB-O], Stop solution [STOP], and Dilution buffer [DIL] are interchangeable between ELISA-VIDITEST kits, unless otherwise noted in the kit instructions.
- d. Work aseptically to avoid microbial contamination of samples and reagents.
- e. When collecting, diluting, and storing reagents, be careful not to cross-contaminate them or contaminate them with enzymatic activity inhibitors.
- f. 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.
- g. 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 Chapter 7
 - * 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
- h. 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.
- i. Neutralize liquid waste containing Stop solution (sulfuric acid solution) with 4% sodium bicarbonate solution before disposal.
- j. 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.
- k. 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.
- l. 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.
- m. All reagents and packaging material must be disposed of in accordance with applicable legislation.
- n. 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.

12. SAFETY PRECAUTIONS

Calibrator [CAL], Positive control human serum [PC], Negative control human serum [NC] and Dilution buffer [DIL] are preserved with ProClin 300 (a mixture of 5-Chloro-2-methyl-4-isothiazolin-3-one and 2-Methyl-2H-isothiazol-3-one (3:1)). Therefore, the following warnings and safety precautions apply to these solutions:

	Warning	H317	May cause an allergic skin reaction.
		H411	Toxic to aquatic life with long lasting effects.
		P280	Wear protective gloves/protective clothing/ protective glasses/ face protection.
		P302+P352	OF ON SKIN: Wash with plenty of water.
		P333+P313	If skin irritation or rash occurs: Get medical advice/attention.
		P362+P364	Take off contaminated clothing and wash it before reuse.

The anti-IgG Px conjugate [CONJ] contains N-methyl-2-pyrrolidone. Therefore, the following warnings and precautions apply to this solution:

	Danger	H360D	Warning: May damage the unborn child.
		P202	Do not use until you have read and understood all safety instructions.
		P280	Wear protective gloves/protective clothing/ protective glasses/ face protection.
		P308+P313	If exposed or concerned: Get medical advice/attention.
		P501	Dispose of contents/container in accordance with local regulations.

Further information can be found in the safety data sheet.

13. STORAGE AND EXPIRATION

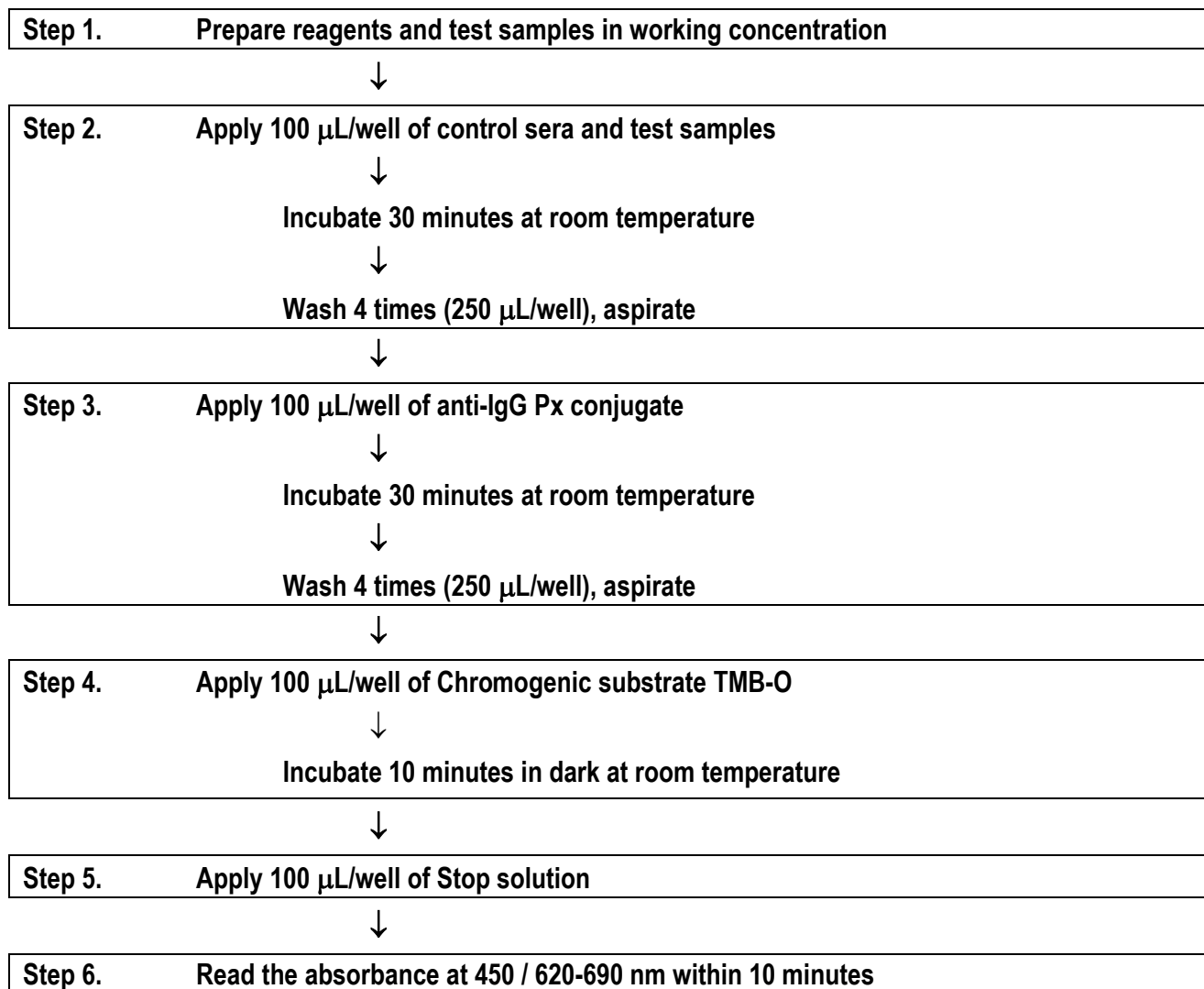
It is recommended to use the kit within three months after opening.

- Store the kit and the kit reagents at +2 °C to +10 °C, in a dry place and protected from the light. Under these conditions, the expiration period of the entire kit is indicated on the central label on the kit package, the expiration date of the individual components is indicated on their package.
- Put unused strips back in the package and seal or close tightly in a zippered bag with desiccant.
- The kits are transported refrigerated in thermal bags, transport time up to 72 hours has no influence on expiration. If, upon receipt of the kit, you notice serious damage to the packaging of any component of the kit, inform the manufacturer immediately.
- Store unused test samples undiluted, aliquoted and frozen at -18 °C to -28 °C. Frequent freezing and thawing is not recommended. If you store samples at + 2 °C to + 10 °C, then test them within one week.
- Test sample solutions at the working concentration cannot be stored. Always prepare them fresh.

14. USED SYMBOLS

Symbol	Explanation
	number of tests
	Conformité Européenne – product meets the requirements of European legislation
	diagnostics <i>in vitro</i>
$\pm\sigma$	standard deviation
CV	coefficient of variation
OD	optical density
	manufacturer
	expiration
	lot of kit
	storage at +2 °C - +10 °C
°C	Celsius degree
%	percentage
n	number of tested samples
A	value of a certain sample
	read the package leaflet
	catalog number

15. TEST SCHEME



References:

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