HSV-1/2-IgM-ELA Test PKS medac

English

MANUFACTURER

medac
Gesellschaft für klinische
Spezialpräparate mbH
Fehlandtstraße 3
D-20354 Hamburg

MARKETING

medac
Gesellschaft für klinische
Spezialpräparate mbH
Geschäftseinheit Diagnostika
Theaterstraße 6
D-22880 Wedel

Phone: ++49/ 4103/ 8006-351
Fax: ++49/ 4103/ 8006-359

ORDERING ADDRESS

Phone: ++49/ 4103/ 8006-111
Fax: ++49/ 4103/ 8006-113

HSV-1/2-IgM-ELA Test PKS medac
Enzyme immunoassay with Pipetting Control System for the detection of IgM antibodies to Herpes simplex virus 1 and 2 (HSV-1/2) in serum

Cat. no.: 104-PKS

FOR IN VITRO DIAGNOSTIC USE ONLY

INTRODUCTION

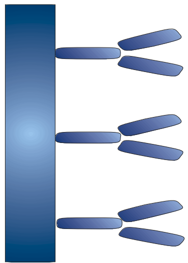
The herpes simplex virus (HSV) belongs to the family of human pathogenic *herpesviridae*. There are two types, HSV-1 and HSV-2, that share 85 % of genetic homology. The envelope glycoprotein G determines type-specificity of the virus. Lifelong latent persistence in the organism after primary infection is typical for HSV. Reactivations occur in about 50 % of latently infected and more frequently in immunocompromised people. Heterologous as well as homologous secondary infections are possible. HSV are common worldwide. In Germany the prevalence of HSV-1 antibodies is more than 90 % in adults whereas nearly 15 % possess antibodies to HSV-2 with increasing tendency.

Virus transfer occurs via mucosa or skin contact. Infections manifest as oro-facial (HSV-1) or genital (mainly HSV-2) herpes. Type-specific localisation of the disease is not obligatory because the two virus types may cause infections in both body regions.

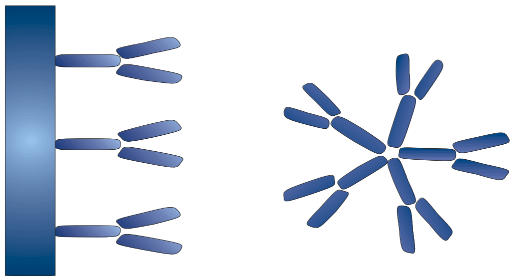
Diagnosis of primary or reactivated HSV infection in the symptomatic/active phase of disease is carried out in most cases by clinical signs or by direct detection of the pathogen. HSV laboratory diagnosis is mainly used in cases of exanthema with unclear genesis, suspected herpes encephalitis, generalised infection of immuno-compromised or newborn patients and genital infection during pregnancy. The determination of antibodies is used preferably for confirmation of immunity as well as for differentiation of early phase of infection from recurrence. The detection of specific IgM antibodies indicates virus activity. IgM is not reliable for the differentiation of primary infection from reactivation.

The HSV-1/2-IgM-ELA Test PKS medac detects IgM antibodies in serum directed against HSV-1 and/or HSV-2. The test can be fast and easily carried out. The µ-capture and ELA principles allow a highly specific and sensitive diagnostic interpretation.

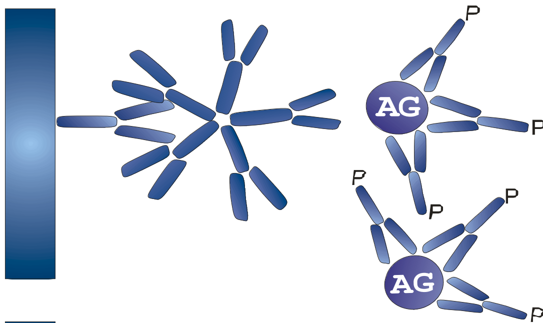
TEST PRINCIPLE



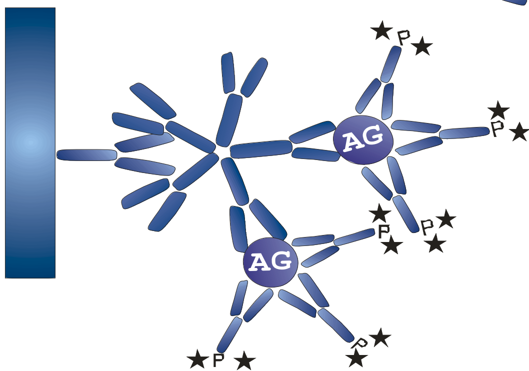
The plate is coated with anti-human IgM immunoglobulin.



IgM of the specimen is selectively bound to the wells.



The complex of mAb-peroxidase-conjugate and HSV-antigen binds to anti-HSV-1/2 IgM specific antibodies (AG = antigen, P = peroxidase).



Incubation with TMB-substrate (*). The reaction is stopped by the addition of sulfuric acid. The absorption is read photometrically.

Advantages of the test

- No unspecific reactions and no false positive results caused by rheumatoid factors.
- No blocking of IgM antibodies by high IgG titer.
- The Pipetting Control System allows to monitor visually each pipetting step through colour changes.
- The breakable microwell stips permit efficient use of the test.
- Suitable for automation on open ELISA devices.

KIT CONTENTS
Cat. no.: 104-PKS

1. **MTP**
   Microplate: 12 x 8 wells (with frame and desiccant vacuum sealed in aluminium bag), breakable, U-form, coated with goat anti-human IgM immunoglobulin, BSA and pH indicator, ready to use.

2. **CONTROL | –**
   Negative control: 1 vial with 1.5 ml, human serum, ready to use, contains BSA, phenol, ProClin™ 300 and gentamycin sulfate.

3. **CONTROL | +**
   Positive control: 1 vial with 1.5 ml, human serum, ready to use, contains FCS, BSA, phenol, ProClin™ 300 and gentamycin sulfate.

4. **WB**
   Wash buffer: 1 bottle with 100 ml, PBS/Tween (10x), pH 7.2 - 7.4, contains ProClin™ 300.

5. **VIR-DIL**
   Sample diluent: 1 bottle with 110 ml, PBS/Tween/BSA, pH 7.2 - 7.4, ready to use, contains ProClin™ 300.

6. **ANTIGEN-DIL**
   Antigen diluent: 1 vial with 14 ml PBS/Tween, ready to use, contains ProClin™ 300.

7. **ANTIGEN**
   Antigen: 6 vials with 2.0 ml each, HSV-antigen/BSA/FCS, lyophilised.

8. **CON**
   Conjugate: 1 vial with 0.35 ml, anti HSV-IgG antibodies, monoclonal (mouse), HRP-conjugated, ready to use, stained red, contains BSA, phenol, ProClin™ 300 and gentamycin sulfate.

9. **TMB**
   TMB-substrate: 1 vial with 10 ml, ready to use.

10. **STOP**
    Stop solution: 2 vials with 11 ml each, 0.5 M sulfuric acid (H_2SO_4), ready to use.

1. STORAGE AND STABILITY

Material/Reagent	**State**	**Storage**	**Stability**
Test kit	unopened	2...8 °C	until expiry date
Microplate	opened	2...8 °C in bag with desiccant	6 weeks
Controls	opened	2...8 °C	6 weeks
Wash buffer	diluted	2...8 °C	6 weeks
Sample diluent	opened	2...8 °C	6 weeks
Antigen diluent	opened	2...8 °C	6 weeks
Conjugate	opened	2...8 °C	6 weeks
Antigen-conjugate mixture	ready to use	RT	6 hours
TMB-substrate	opened	2...8 °C	6 weeks
Stop solution	opened	2...8 °C	until expiry date

Do not use the reagents after the expiry date.

2. REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED

2.1. Water for injection (H_2O redist.). Use of deionised water can disturb the test procedure.

2.2. Adjustable micropipettes.

2.3. Clean glass or plastic containers for dilution of wash buffer and specimen.

2.4. Suitable device for microplate washing (e.g. multistepper or ELISA washer).

2.5. Incubator for 37 °C.

2.6. Microplate reader with filters for 450 nm and 620 - 650 nm.

3. PREPARATION OF THE REAGENTS

Before starting the test procedure all kit components must be equilibrated to room temperature.

Calculate the number of wells required.

3.1. Microplate

The aluminium bag has to be tightly resealed together with the desiccant after each removal of wells. Storage and stability of the wells are indicated below point 1.

Note: The microplate wells have a light green colour. Possibly occurring greenish brown stains inside the wells are due to the production process and do not influence the test performance.

3.2. Wash buffer

Mix one volume of wash buffer (10x) with nine volumes of water for injection (e.g. 50 ml wash buffer (10x) with 450 ml water). 10 ml of diluted wash buffer are needed for eight wells.

Crystals in the wash buffer (10x) have to be dissolved by warming (max. 37 °C) and/or stirring at RT.

3.3. Antigen-conjugate mixture

Reconstitute the lyophilised antigen with 2.0 ml **antigen diluent** each. Mix gently and take care that particles sticking to the closure are also dissolved. Add 50 µl conjugate to 2.0 ml antigen 60 min before use.

After reconstitution of the antigen and addition of the conjugate, the antigen-conjugate mixture has a red colour and is ready to use.

The ready-to-use antigen-conjugate mixture can be used at room temperature for 6 hours (see 1.).

Do not mix test specific reagents (microplate, controls, conjugate, antigen, antigen diluent) from different kit lots. In contrast to that sample diluent, wash buffer, TMB-substrate and stop solution are generally exchangeable in all virological ELISA medac.

Reagents from other manufacturers must not be used in general.

Valid and reproducible results are only obtained if the test procedure is precisely followed.

4. SPECIMEN

4.1. The test is suitable for serum.

4.2. Pretreatment of sera, e.g. inactivation, is not necessary. However, they should neither be contaminated with microorganisms nor contain any red blood cells.

4.3. Sera have to be diluted 1:100 with sample diluent.

5.A. TEST PROCEDURE

5.1. Cut the aluminium bag above the zip fastener and take out the required number of microplate wells (see 3.1.).

Microplate wells are ready to use and do not have to be prewashed.

5.2. Leave well A1 empty as blank (see 6.A.). Add 50 µl each of the diluted sample as well as negative control and positive control in duplicates to the wells.

After pipetting the samples (pH neutral or basic fluid) the wells turn blue. A missing colour change in one well indicates that no sample or control has been added.

If necessary, the microplate wells can be kept up to 30 min at RT before proceeding.

5.3. Incubate the microplate wells for 60 min (± 5 min) at 37 °C (± 1 °C) in a humid chamber or sealed with incubation cover foil.

5.4. Prepare the antigen-conjugate mixture (see 3.3.).

5.5. After incubation wash the microplate wells three times with 200 µl wash buffer per well. Pay attention that all wells are filled. After washing tap microplate wells on filter paper.

Do not allow the wells to dry out! Proceed immediately!

5.6. Add antigen-conjugate mixture (coloured red) to each well (except A1).

50 µl of the antigen-conjugate mixture have to be pipetted into the wells if the test is done manually.

Please note:
When working with automated devices, 60 µl of the antigen-conjugate mixture have to be pipetted into each well due to a higher evaporation in the incubation chambers of the devices.

The suitability of the test for automated devices was confirmed during the evaluation of the test. Nevertheless we recommend to verify the compatibility of the test with the devices used in the lab.

5.7. Incubate again for 60 min (± 5 min) at 37 °C (± 1 °C) in a humid chamber or sealed with incubation cover foil.

5.8. After incubation wash microplate wells again (see 5.5.).

5.9. Add 50 µl of TMB-substrate to each well (also A1) and incubate for 30 min (± 2 min) at 37 °C (± 1 °C) in a humid chamber or sealed with incubation cover foil in the dark. Positive samples turn blue.

5.10. Stop the reaction by adding 100 µl of stop solution to each well (also A1). Positive samples turn yellow.

Clean microplate wells from underneath before the photometric reading and take care that there are no air bubbles in the wells.

The reading should be done within 15 min after adding the stop solution.

5.B. TABLE FOR THE TEST PROCEDURE

	Blank (A1)	Negative control	Positive control	Sample
Negative control Positive control Sample	– – –	50 µl – –	– 50 µl –	– – 50 µl
Incubate for 60 min at 37 °C, wash 3x with 200 µl wash buffer				
Antigen-conjugate mixture	–	50/60 µl*)	50/60 µl*)	50/60 µl*)
Incubate for 60 min at 37 °C, wash 3x with 200 µl wash buffer				
TMB-substrate	50 µl	50 µl	50 µl	50 µl
Incubate for 30 minutes at 37 °C in the dark				
Stop solution	100 µl	100 µl	100 µl	100 µl
Photometric reading at 450 nm (ref. 620 - 650 nm)				

*) manual/automatic procedure (see 5.6.)

6.A. CALCULATION OF RESULTS (VALIDITY)

* Read OD values at 450 nm (reference wavelength 620 - 650 nm).

* Subtract the OD value of the blank (well A1) from all other OD values.

* The mean OD value of the **negative control** has to be **< 0.150.**
  The mean OD value of the **positive control** has to be **> 0.450.**

* **Cut-off = mean OD value of the negative control + 0.140**

* **Grey zone = Cut-off ± 10 %**

  Repeat the run if the results do not meet the specification.

6.B. INTERPRETATION OF RESULTS/LIMITATIONS OF THE METHOD

* Samples with OD values below the lower limit of the grey zone are reported as **NEGATIVE.**

* Samples with OD values within the grey zone are reported as **EQUIVOCAL.** These samples should be retested together with a fresh specimen taken 14 days later in order to determine a titer change.

* Samples with OD values exceeding the upper limit of the grey zone are reported as **POSITIVE.**

* The results should always be interpreted in connection with clinical data of the patients as well as the HSV-1/2-IgG, the HSV-2-IgG results and additional diagnostic parameters.

  In case of suspected primary HSV-1/2 infection during pregnancy further diagnostic, e.g. PCR, has to be performed.

* Cross reactions, caused by antibodies against other herpes viruses, cannot be excluded in single cases.

* Hemolytic sera do not influence the test results.

7. PERFORMANCE CHARACTERISTICS

We determined the following performance characteristics during the diagnostic evaluation.

7.A. SENSITIVITY AND SPECIFICITY

468 sera (270 from the lab Prof. Dr. Enders and Colleagues, Stuttgart, and 198 sera of blood donors from Hanover and Suhl) were measured in comparison to the predefinition during the diagnostic evaluation. The predefinition was assessed with two different ELISA as reference.

The results are shown in the following table:

HSV-1/2-IgM-ELA Test PKS medac		Predefinition		
		negative	cut-off	positive
	negative	409	0	9
	cut-off	0	0	0
	positive	0	0	50

Specificity = 100 %
Sensitivity = 84.7 %

Concordance: 98.1 %

7.B. PRECISION

Sample	**Intra-assay variation**				**Sample**	**Interassay variation**			
	mean OD	**SD**	**CV (%)**	**n**		**mean OD**	**SD**	**CV (%)**	**n**
NC	0.038	0.003	8	22	**NC**	0.050	0.011	22	11
BC	0.313	0.008	3	22	**BC**	0.360	0.036	10	11
PC	0.562	0.017	3	22	**PC**	0.705	0.088	12	11
N° 1	0.035	0.002	6	22	**N° 6**	0.033	0.007	21	11
N° 2	0.073	0.005	7	22	**N° 7**	0.098	0.013	13	11
N° 3	0.417	0.017	4	22	**N° 8**	0.505	0.054	11	11
N° 4	0.609	0.020	3	22	**N° 9**	0.734	0.061	8	11
N° 5	1.257	0.027	2	22	**N° 10**	1.365	0.103	8	11

NC = negative control; BC = weak positive control (not included in the kit);
PC = positive control

GENERAL HANDLING ADVICES

* To avoid cross contamination do not exchange the vials and their screw caps.

* The reagents have to be sealed immediately after use to avoid evaporation and microbial contamination.

* After use, the reagents have to be stored as indicated to guarantee the shelf life.

* After use, all components of the testkit should be stored in the original package, in order to avoid mixing up the reagents of other test systems or lots (see also 3.).

HEALTH AND SAFETY INFORMATION

* The local occupational safety and health regulations have to be regarded.

* Reagents of human origin have been tested and found to be negative for HBsAg, for antibodies to HIV-1/2 and to HCV. Nevertheless, it is strongly recommended that these materials as well as those of animal origin (see kit contents) should be handled as potentially infectious and used with all necessary precautions.

DISPOSAL CONSIDERATIONS

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.

Date of issue: 15.03.2010

REFERENCES

Ashley, R.L., Wald, A.: Genital herpes: Review of the epidemic and potential use of type-specific serology. Clin Microbiol Rev 12(1), 1-8 (1999)

Bergström, T., Trybala, E.: Antigenic differences between HSV-1 and HSV-2 glycoproteins and their importance for type-specific serology. Intervirology 39,176-184 (1996)

Kimberlin, D.W.: Neonatal Herpes simplex Infection. Clin Microbiol Rev 17(1), 1-13 (2004)

Kriebs, I.M.: Understanding Herpes simplex virus: Transmission, diagnosis and considerations in pregnancy management. Midwifery Womens Health 53(3), 202-208 (2008)

Mertens, T., Haller, O., Klenk, H.-D. (Hrsg.): Diagnostik und Therapie von Viruskrankheiten. 126-137, Urban & Fischer Verlag (2004)

Petersen. E.E., Doerr, H.W., Gross, G., Petzoldt, D., Weissenbacher, E.R., Wutzler, P.: Der Herpes genitalis. Dt Ärztebl 96, Heft 38, A-2358-2364 (1999)

Sauerbrei, A., Wutzler, P.: Herpes simplex and varicella-zoster virus infections during pregnancy. Current concepts of prevention, diagnosis and therapy. Part 1: Herpes simplex virus infections. Med Microbiol Immunol 196(2), 89-94 (2007)

Wutzler, P., Sauerbrei, A., Eichhorn, U.: Virologische Diagnostik der Herpes simplex-Virus-Enzephalitis. Mikrobiologe 9, 11-15 (1999)

Wutzler, P., Doerr, H.W., Färber, I., Eichhorn, U., Helbig, B., Sauerbrei, A.: Seroprevalence of herpes simplex virus type 1 and type 2 in selected German populations - relevance for incidence of genital herpes. J Med Virol 61, 201-207 (2000)