

HHV-8 IgG EIA, Cat No: V19HHV8, HHV-415-02 09/09



**Cat No:** V19HHV8  
**Format:** 96 well EIA



## **Human Herpesvirus-8 IgG EIA**

An enzyme immunoassay for the qualitative detection of Human Herpesvirus-8 IgG antibodies in human serum and plasma.

**FOR RESEARCH USE ONLY  
NOT FOR USE IN  
DIAGNOSTIC PROCEDURES**

## Table of Contents

Intended Use

Introduction

Assay Principle

Precautions

*Safety*

*Procedural*

Kit Components

*Materials Provided*

*Additional Materials Required*

Storage and Stability

Specimen Collection and Storage

Reagent and Specimen Preparation

Assay Procedure

Interpretation of Results

Quality Control Criteria

Limitations of Use

Expected Values

Summary of Human Herpesvirus-8 IgG EIA Procedure

Bibliography

Interpretation of Symbols

## **Intended Use**

The Human Herpesvirus-8 (HHV-8) IgG bi-peptide Enzyme Immunoassay is intended for the qualitative detection of IgG antibodies to HHV-8 lytic antigens in human serum or plasma.

## **Introduction**

Human Herpesvirus-8 (HHV-8) is also known as Kaposi's Sarcoma-associated Herpesvirus (KSHV). The virus is classified as a gamma herpesvirus (genus rhadinovirus) and resembles EBV in its tropism for B cells and ability to exist in a latent state. There is now very strong epidemiological evidence for the causative role of HHV-8 in the pathogenesis of Kaposi's Sarcoma (KS) <sup>(1)</sup>. HHV-8 is detectable in all forms of the disease: Classic KS (a rare malignancy occurring in elderly Mediterranean men), African endemic KS, Transplant-associated KS and AIDS associated KS. In HIV positive patients, HHV-8 antibodies have been shown to precede and predict the development of KS <sup>(2)</sup>. Transmission through sexual contact plays an important part in the spread of HHV-8 among homosexual men <sup>(3)</sup>. The seroprevalence of HHV-8 among blood donors ranges from 5 -10% in the United States and N. Europe <sup>(4)</sup>, 10 - 35% in Italy and Mediterranean countries <sup>(5)</sup>, to more than 50% in many African populations <sup>(6)</sup>. HHV-8 has also been associated with body cavity lymphomas, also called primary effusion lymphomas (PEL), multi-centric Castleman's disease (MCD), non-Hodgkin's lymphoma and multiple myeloma <sup>(7)</sup>. At present, HHV-8 infection can be diagnosed by PCR analysis and by immunological assays, e.g. IFA and ELISA. However, HHV-8 DNA can be detected in peripheral blood cells from only about half of infected persons with the use of standard PCR assays <sup>(9-12)</sup>. Since PCR detection systems appear to exhibit low sensitivity when DNA from peripheral blood cells is used as a template, serological assays have proved more useful for epidemiology studies and diagnosis of HHV-8 infection, particularly for detecting previous exposure to the virus <sup>(8,9)</sup>. The Biotrin HHV-8 IgG EIA kit is based on a synthetic peptide mix which allows for the detection of antibodies to lytic HHV-8 viral proteins.

## **Assay Principle**

The BIOTRIN HHV-8 ELISA is a direct EIA based on the binding of HHV-8 specific antibodies to lytic peptide antigens coupled to microtitre test strips. Specifically bound antibodies are detected by an anti-human IgG peroxidase conjugate and a subsequent substrate reaction. The use of lytic peptide epitopes derived from different viral proteins ensures both a high sensitivity and specificity. There is no detectable cross-reactivity with HIV.

## Precautions

### *Safety*

- FOR RESEARCH USE ONLY - NOT FOR USE IN DIAGNOSTIC PROCEDURES
- This kit is intended for use by qualified laboratory staff only.
- Reagents marked with \*\* are considered POTENTIALLY BIOHAZARDOUS MATERIAL. Each donor unit used in the preparation of the positive control, cut off calibrator and negative control was tested by an FDA-cleared method for HBsAg and antibodies to HIV and HCV and found to be negative. However, because no test method can offer complete assurance that infectious agents are absent, all these reagents and all patient specimens should be handled in accordance with established safety procedures.
- Some reagents contain Kathon™ CG, which may be corrosive. Stop Solution contains sulphuric acid, which is also corrosive. Avoid contact with the skin and eyes. If contact occurs rinse off immediately with water and seek medical advice.
- Some reagents contain Thiomersal, which may be toxic if ingested.
- The substrate contains TMB which may irritate the skin and mucous membranes. Any substrate that comes in contact with the skin should be rinsed off with water.
- Dispose of all clinical specimens, infected or potentially infected material in accordance with good laboratory practice. All such materials should be handled and disposed of as though potentially infectious.
- Residues of chemicals, preparations and kit components are generally considered as hazardous waste. All such materials should be disposed of in accordance with established safety procedures.
- Wear protective clothing, disposable latex gloves and eye protection while handling specimens and performing the assay. Wash hands thoroughly when finished.
- Do not pipette materials by mouth and never eat or drink at the laboratory workbench.

***Procedural***

- Performing the assay outside the time and temperature ranges provided may produce invalid results. Assays not falling within the established time and temperature ranges must be repeated.
- Do not use kit or individual reagents past their expiry date.
- Do not use contaminated samples or reagents.
- Do not mix or substitute reagents from different kit lot numbers.
- Deviation from the protocol provided may cause erroneous results.
- Allow all reagents to come to room temperature (20 - 25°C) and mix well prior to use.
- Avoid leaving reagents in direct sunlight and/or above 2-8°C for extended periods.
- High Quality distilled or deionised water is required for the Wash Solution.
- Always use clean, preferably disposable, glassware for all reagent preparation.
- Care must be taken not to contaminate components and always use fresh pipette tips for each sample and component.
- Remove only the volume of conjugate required for the assay. Do not pour unused reagent back into the bottle or pipette directly from the bottle. If so contamination may occur.
- Reagent delivery should be aimed at midpoint of the side of the wells, taking care not to scratch the side with the pipette tip.
- Do not allow the wells to dry up at any stage during the assay procedure.
- Always keep the upper surface of the wells free of droplets. Drops should be gently blotted dry on completion of the procedural step.
- Ensure that the bottom surface of the plate is clean and dry before reading.
- Before commencing the assay an identification and distribution plan should be established.
- Do not heat-inactivate sera.
- Do not remove the plate from its protective pouch until ready to use.

## Kit Components

### Materials Provided

1. Coated ELISA plate  

PLA	IgG
-----	-----

  
 12 x 8 wells streptavidin coated biotinylated HHV-8 lytic peptides
2. Positive Control\*\* (Red Cap Colour)  

CONTROL	+	IgG
---------	---	-----

  
 1 x 2 mL of prediluted positive human serum or plasma in a stabilising buffer (containing 0.01% sodium azide, 0.01% Thiomersal)
3. Negative Control\*\* (Green Cap Colour)  

CONTROL	-	IgG
---------	---	-----

  
 1 x 2 mL of prediluted negative human serum or plasma in a stabilising buffer (containing 0.01% sodium azide, 0.067% Kathon™ CG)
4. Cut-off-Calibrator\*\* (Brown Cap Colour)  

CAL
-----

  
 1 x 2 mL of prediluted weakly positive human serum or plasma in a stabilising buffer (containing 0.01% sodium azide, 0.067% Kathon™ CG)
5. Enzyme Conjugate Diluent  

CONJ	ENZ	DIL
------	-----	-----

  
 1 x 17 mL of IgG enzyme conjugate diluent (containing 0.01% gentamicin sulphate, 0.01% Thiomersal)
6. Enzyme Conjugate Concentrate  

CONJ	ENZ	10X
------	-----	-----

  
 1 x 1.7 mL of IgG enzyme conjugate concentrate (containing 0.01% gentamicin sulphate, 0.01% Thiomersal).
7. Sample Diluent (Ready-to-use)  

DIL	SPE	1X
-----	-----	----

  
 1 x 110 mL PBS buffer containing stabilisers and Kathon™ CG (0.067%).
8. Wash Concentrate  

BUF	WASH	25X
-----	------	-----

  
 1 x 55 mL of concentrated (25X) Tris buffer with Tween 20 (2.75%) and Kathon™ CG (0.067%).
9. Substrate  

SUBS	TMB
------	-----

  
 1 x 17 mL of tetramethylbenzidine (TMB) solution

10. Stop Solution

SOLN	STP
------	-----

1 x 17 mL of 0.5M H<sub>2</sub>SO<sub>4</sub>

11. Instructions for Use



\*\* Potentially Biohazardous Material

Kathon™ CG is a registered trademark of Rohm and Haas Company.

***Additional Materials Required***

- Serum collection equipment
- High quality distilled or deionised water
- Clean volumetric labware
- Test tubes or equivalent for sample preparation
- Graduated cylinders
- Accurate pipettes, micropipettes and disposable tips to deliver 10 uL, 100 uL, 1 mL and 5 mL volumes
- Plastic lid or sealing tape for microwell plate
- Timer
- Manual or automatic washing device
- 35 - 39°C incubator
- Paper towels or absorbent paper
- ELISA plate reader with 450nm filter (additional 630 – 650nm filter is optional)

**Storage and Stability**

- The kit is stable until the expiry date indicated on the outer box label, provided it is stored between 2-8°C.
- 8-well strips should be stored in the resealable pouch along with the sachets of desiccant.
- All unused components should be returned to 2-8°C storage immediately after use.
- Reconstituted Wash Solution is stable for 1 month when stored at 2-8°C.

## **Specimen Collection and Storage**

Either serum or plasma can be used in the Biotrin HHV-8 IgG EIA. Once collected by venipuncture, blood should be allowed to clot at room temperature (20-25°C) followed by centrifugation at 1500 x g for 10 minutes. If not for immediate testing within 8 hours, the serum or plasma can be placed at 2-8°C for up to 2-3 days or frozen at -20°C if extended storage or shipment is required (samples are stable at -20°C for at least 1 year). Citrated plasma is compatible with the test procedure. Microbially contaminated sera should not be used for testing. Finally, test specimens should not be subjected to repeated freeze-thaw cycles.

**Note:** It is recommended that samples are not heat inactivated.

## **Reagent and Specimen Preparation**

### ***Reagent Preparation***

- Reagent volumes are based on singleton sample testing.
- Wash Solution  
For each 8-well Strip add 4 mL of Wash Concentrate to 96 mL of deionised water. Prepared reagent is stable for 1 month if stored at 2-8°C.
- Enzyme Conjugate preparation  
For each 8-well Strip add 100 uL of Enzyme Conjugate to 900 uL of enzyme conjugate diluent. Prepared solution should not be stored.

All remaining reagents are supplied ready - to - use and are at working dilution.

### ***Specimen Preparation***

For each sample dispense 1ml of Sample Diluent into a labelled test tube or equivalent. Add 10 uL of serum or plasma sample and mix.

**Note:** Diluted samples should not be stored. If a repeat test is required a fresh preparation should be used.

### Assay Procedure

1. Allow all components to equilibrate to room temperature (20-25°C) before use.
2. Determine the number of wells required. Establish an identification and distribution plan for controls and samples as indicated in Figure 1 (below). The first strip is suitable for testing 2 patient specimens, each additional strip allows for testing of a further 8 patient specimens.

**Figure 1.** Strip 1

A	Negative Control
B	Negative Control
C	Positive Control
D	Positive Control
E	Cut-Off-Calibrator
F	Cut-Off-Calibrator
G	Patient No. 1
H	Patient No. 2

Remove the desired number of wells, place in a plastic frame and cover with a plastic lid/sealant tape. Return the remaining strips to the pouch and reseal along with desiccant.

3. Prepare Wash Solution (see "Reagent and Specimen Preparation").
4. Prepare patient specimen (see "Reagent and Specimen Preparation").
5. Remove cover from strips and pipette 100 uL, in duplicate, of the ready-to-use Negative Control, Cut-Off-Calibrator, Positive Control and 100 uL in singleton of the prepared patient specimens to the wells.
6. Cover the wells with a plastic lid/sealing tape and incubate for 30 minutes at 35 - 39°C.
7. Remove cover and wash each well 4 times with Wash Solution (250 – 300 uL).
8. After washing firmly tap the plate against an absorbent paper towel.
9. Prepare Enzyme Conjugate (see "Reagent and Specimen Preparation").
10. Pipette 100 uL of the prepared IgG Enzyme Conjugate into all wells immediately after the wash step is completed.
11. Cover the wells with a plastic lid/sealing tape and incubate for 30 minutes at 35 - 39°C.
12. Remove cover and wash each well 4 times with Wash Solution (250 – 300 uL).
13. After washing firmly tap the plate against an absorbent paper towel.
14. Pipette 100 uL of TMB Substrate into all wells immediately after the wash step is completed.
15. Cover the wells with a plastic lid/sealing tape and incubate for exactly 30 minutes at 35 - 39°C.
16. Pipette 100 uL of Stop Solution into all wells and mix. Ensure that each addition is in the same sequence and time interval as the addition of Substrate.
17. Read immediately with an ELISA plate reader.

**Note:** Dual wavelength reading is recommended at 450nm with 630nm as the reference wavelength. If this function is not available on the ELISA plate reader use a single wavelength reading at 450nm.

### **Interpretation of Results**

The presence or absence of anti-HHV-8 IgG is determined in relation to the Cut – Off Calibrator (COC).

#### ***Cut – Off Calibrator Value***

- 1) Determine the COC value by assaying the Cut-Off Calibrator in each assay in duplicate.
- 2) Determine the mean OD value, this value is the COC value and is to be used to determine index values.
- 3) An index value is calculated by dividing the sample/control absorbance by the COC value.

#### ***Interpretation (1): Absorbance***

Samples with an absorbance reading greater than the COC x 1.2 are considered reactive (positive) for anti-HHV-8 IgG.

Samples with an absorbance reading less than the COC x 0.8 are considered nonreactive (negative) for anti-HHV-8 IgG.

Samples with an absorbance reading greater than or equal to COC x 0.8 and less than or equal to COC x 1.2 are equivocal.

#### ***Interpretation (2): Index Value***

Data comparison between different assay runs is facilitated by using an index value whereby sample absorbance is expressed relative to the assay cut-off calibrator. In this case, an index value <0.8 or >1.2 indicates sample negativity or positivity, respectively. Equivocality is indicated if the index value is in the range 0.8-1.2 inclusive.

$$\text{Index} = \frac{\text{Control/Sample absorbance}}{\text{Mean Cut-off Calibrator (COC) absorbance}}$$

Samples which are neither reactive (positive) or non-reactive (negative) are considered equivocal and should be re-tested. If the re-test result is equivocal then a second sample should be collected 7-14 days later. An equivocal result with the second sample may be considered unreactive (negative) for anti-HHV-8 IgG, however if recent infection is suspected, it may be confirmed by testing on an alternative method.

### **Quality Control Criteria**

The Positive Control and Negative Control must always be included to determine the validity of test results. Results of an assay are considered valid if the following criteria are met:

1. The mean index of the Positive Control is greater than or equal to an index of 1.2
2. The mean index of the Negative Control is less than an index of 0.8.

If the above criteria are not met the assay is considered invalid and must be repeated.

### **Limitations of Use**

- **FOR RESEARCH USE ONLY - NOT FOR USE IN DIAGNOSTIC PROCEDURES**
- Results must be correlated with the patient's clinical and epidemiological profile and other clinical laboratory results in detecting HHV-8 infection.
- A non-reactive (negative) result does not exclude the possibility of HHV-8 infection. The development of a detectable antibody response may occur some days after infection. In the case of suspected HHV-8 infection a negative result should be followed with a repeat test two weeks later.
- Insufficient data is available to support the interpretation of results of tests performed on other body fluids or pools of sera/plasma.
- Test performance may be affected by deviation from the procedure, interpretation or recommended precautions.
- Assay performance has been validated based on testing controls in duplicate and samples in singleton.

### **Expected Values**

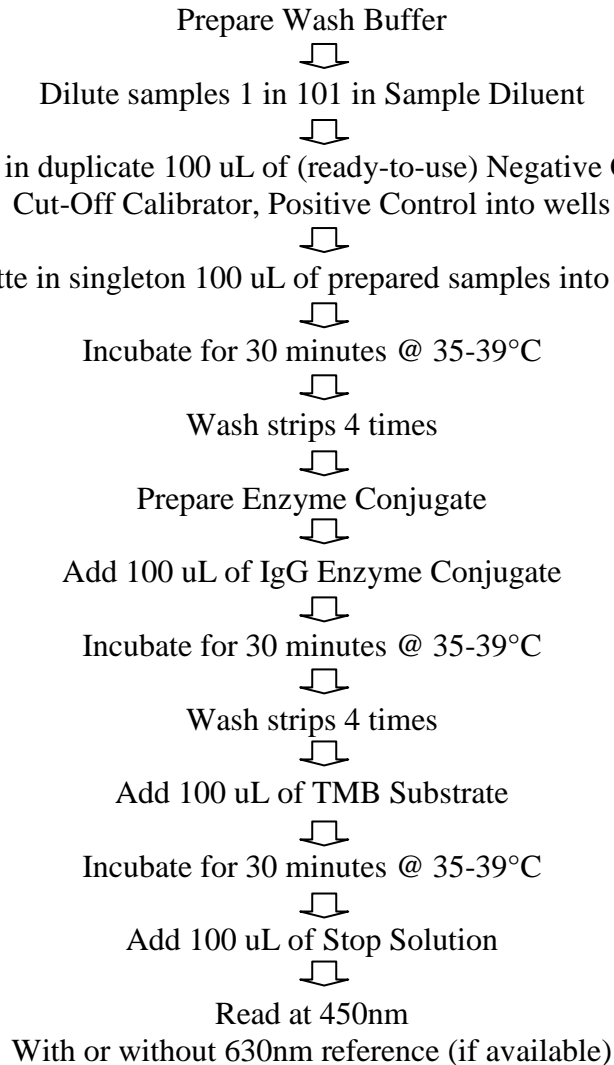
#### ***Seroprevalence***

Disease prevalence is usually determined after extensive testing for antibody levels, in any given population, according to age, sex, geographical location, and socioeconomic status.

The seroprevalence of HHV-8 among blood donors ranges from 5-10% in the United States and N. Europe<sup>(4)</sup>, 10-35% in Italy and Mediterranean countries<sup>(5)</sup>, to more than 50% in many African populations<sup>(6)</sup>.

**Summary of Human Herpesvirus-8 IgG EIA Procedure**

Please read the entire product instruction leaflet before starting the assay. This summary is for quick reference only.



**Bibliography:**

1. The Role of HHV-8 in Kaposi's Sarcoma. Neipel F and Fleckenstein B. *Semin Cancer Biol.* 1999; 9(3):151-164.
2. Seroconversion to antibodies against Kaposi's sarcoma-associated herpesvirus related latent nuclear antigens before the development of Kaposi's sarcoma. Gao et al. *N Engl. J of Med* 1996; 335:233-41.
3. Risk factors for Human Herpesvirus 8 seropositivity and seroconversion in a cohort of homosexual men. Dukers et al. *American J of Epidemiology* 2000; 151, 213-24.
4. KSHV antibodies among Americans, Italians and Ugandans with and without Kaposi's sarcoma. Gao SJ et al. *Natl Med* 1996; 2:925-8.
5. Human herpesvirus 8 seroprevalence in blood donors and lymphoma patients from different regions of Italy. Whitby D et al. *J Natl Cancer Inst* 1998;90:395-7.
6. Antibodies against human herpesvirus 8 in black South African patients with Cancer. Sitas F et al. *N Engl. J of Medicine* 1999; 340:1863-71.
7. Kaposi's sarcoma after renal transplantation. Camille Frances. *Nephrol Dial Transplant* 1998; 13: 2768-2773.
8. The Role of Kaposi's sarcoma-associated herpesvirus (KSHV / HHV-8) in lymphoproliferative diseases. Cesarman E, Knowles, D. M. *Cancer Biology* 1999; 9, 165-174.
9. Detection of Human Herpesvirus 8 DNA in kaposi's sarcoma lesions and peripheral blood of human immunodeficiency virus-positive patients and correlation with serological measurements. Smith M.S. et al. *J of Infect Dis* 1997; 176:84-93.
10. Comparison of serological assays and PCR for Diagnosis of Human herpesvirus 8 Infection. Spira TJ et al. *J of Clinical Microbiology* 2000; 38 (6): 2174 – 2180.
11. Kaposi's sarcoma. Karen Antman. *Medical Progress* 2000; 342; 14 1027-1037.
12. Chang et al *Science* 265:1865-1869, 1994.

### Interpretation of Symbols

Batch code



Catalogue Number



Temperature limitation



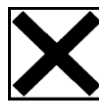
Use by end of



Manufacturer



Harmful if swallowed. Contact  
with acids liberates very  
toxic gases.



Instructions for Use



**Biotrin HHV Product range**

Biotrin International offers a unique portfolio of Human Herpesvirus assays.

Cat #:	Description	Assay Format
V3HHV6	Human Herpesvirus-6 IgG IFA	4 x 10 well slide
V17HHV6	Human Herpesvirus-6 IgM IFA	4 x 10 well slide
V15HHV6	Human Herpesvirus-6 IgG EIA	96 well EIA
V18HHV8	Human Herpesvirus-8 IgG IFA	6 x 10 well slide
V19HHV8	Human Herpesvirus-8 IgG EIA	96 well EIA



Biotrin International Ltd.  
93 The Rise, Mount Merrion  
Co. Dublin  
Ireland  
Tel: +353 (01) 2831166  
Fax: +353 (01) 2831232  
e-mail: [info@biotrin.ie](mailto:info@biotrin.ie)  
[www.biotrin.com](http://www.biotrin.com)

**Document Code:** HHV-415-02-09/09