

Murex HBsAg Version 3

Enzyme immunoassay for the detection of hepatitis B surface antigen in human serum or plasma

Customer Service

For additional product information, please contact your local customer service organization.

This instructions for use must be read carefully prior to use. The instructions for use must be carefully followed. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions for use.

IVD

<http://www.kimhung.vn>

Key to symbols used

REF	List Number	IVD	<i>In Vitro</i> Diagnostic Medical Device
LOT	Lot Number		Store at 2-8°C
	Expiration Date		CAUTION: Consult accompanying documents
	Manufacturer		Consult instructions for use

See **REAGENTS** section for a full explanation of symbols used in reagent component naming.

INTENDED USE

Murex HBsAg Version 3 is a rapid and sensitive enzyme immunoassay for the detection of hepatitis B surface antigen in human serum or plasma.

SUMMARY AND EXPLANATION OF THE TEST

The causative agent of serum hepatitis is hepatitis B virus (HBV) which is an enveloped DNA virus. During infection, HBV produces an excess of hepatitis B surface antigen (HBsAg), also known as Australia antigen, which can be detected in the blood of infected individuals. HBsAg is the first serological marker after infection with HBV appearing one to ten weeks after exposure and two to eight weeks before the onset of hepatitis^{1,2}. HBsAg persists during this acute phase and clears late in the convalescence period. Failure to clear HBsAg within six months indicates a chronic HBsAg carrier state. Blood from individuals in the acute or chronic state is potentially infectious to recipients and should not be transfused. Consequently, potentially infectious samples of serum, EDTA plasma or citrate plasma can be identified.

PRINCIPLE OF THE PROCEDURE

In Murex HBsAg Version 3, the sample is pre-incubated in microwells coated with a mixture of mouse monoclonals specific for different epitopes on the 'a' determinant of HBsAg. Affinity purified goat antibody to HBsAg conjugated to horseradish peroxidase is then added to the sample in the well. During the two incubation steps any HBsAg present in the sample is bound to the well in an antibody-antigen-antibody-enzyme complex. In the absence of HBsAg no conjugate will be bound. After washing to remove sample and unbound Conjugate, a solution containing 3,3', 5,5'-tetramethylbenzidine (TMB) and hydrogen peroxide is added to the wells. Wells which contain HBsAg and hence bound Conjugate will develop a purple colour which is converted to orange when the enzyme reaction is terminated with sulphuric acid.

REAGENTS

DESCRIPTION, PREPARATION FOR USE AND RECOMMENDED STORAGE CONDITIONS

See also **Warnings and Precautions**.



All components must be stored at 2 to 8°C, unless otherwise stated, under which condition they will retain activity until the expiry date of the kit.



COATED WELLS

1. Coated Wells

One plate (9F80-01) or five plates (9F80-05) of 96 wells coated with mouse monoclonal antibody to HBsAg.

Allow the wells to reach room temperature (18 to 30°C) before removal from the bag. Place unused wells in the sealable storage bag provided and return to 2 to 8°C.

SAMPLE DIL

2. Sample Diluent

One bottle containing 16 ml of green/brown buffer containing detergents and proteins of goat and bovine origin. Mix by inversion before use. Contains 0.05% ProClin® 300 preservative.

CONTROL - !

3. Negative Control

One bottle containing 2.5 ml of normal human serum. The serum is diluted in a buffer containing protein of bovine origin. Contains 0.05% Bronidox® preservative.

CONTROL + !

4. Positive Control

One bottle containing 2 ml of inactivated human serum. The serum is diluted in a buffer containing protein of bovine origin. Contains 0.05% Bronidox® preservative.

CONJUGATE

5. Conjugate

One bottle containing 6 ml (9F80-01) or two bottles each containing 16 ml (9F80-05) of horseradish-peroxidase labelled goat antibody to HBsAg in a red buffer containing proteins of bovine and goat origin. Mix by inversion before use. Contains 0.05% ProClin® 300 preservative.

SUBSTRATE DIL

6. Substrate Diluent

One bottle containing 35 ml of a colourless solution of tri-sodium citrate and hydrogen peroxide.

SUBSTRATE CONC

7. Substrate Concentrate

One bottle containing 35 ml of 3,3',5,5'-tetramethylbenzidine (TMB) and stabilisers in a pink solution.

Substrate Solution

To prepare the Substrate Solution add a volume of colourless Substrate Diluent to an equal volume of pink Substrate Concentrate in either a clean glass or plastic vessel. **It is important that this order of addition is followed and that any pipettes and glassware used to prepare Substrate Solution are clean.**

Alternatively, the Substrate Solution may be made by pouring the entire contents of the bottle of Substrate Diluent into the bottle of Substrate Concentrate. One bottle of Substrate Solution provides sufficient reagent for at least five plates - see **Table 1**:

Table 1
Volume of Substrate Concentrate and Substrate Diluent Required

Number of Wells											No of Plates			
8	16	24	32	40	48	56	64	72	80	96	1	2	3	4
Substrate Concentrate (ml)														
0.5	1.0	2.0	2.5	2.5	3.0	3.5	4.0	4.5	4.5	6.0	6	12	18	22
Substrate Diluent (ml)														
0.5	1.0	2.0	2.5	2.5	3.0	3.5	4.0	4.5	4.5	6.0	6	12	18	22

Additional reagent may be required for use with automated systems. Keep away from sunlight. The Substrate Solution should be pink; if it is purple before being used, it should be discarded and fresh Substrate Solution prepared.

The prepared Substrate Solution from this kit may be used interchangeably with that from all other Murex kits which use pink coloured Substrate Concentrate. Ensure that the Substrate Solution is prepared from Substrate Diluent and Substrate Concentrate provided together.

The prepared Substrate Solution is stable refrigerated (2 to 8°C) or at 15 to 25°C for up to two days but must be discarded if crystals have formed.

WASH FLUID

8. Wash Fluid

One bottle containing 125 ml of 20 times working strength Glycine/Borate Wash Fluid. Contains 0.2% Bronidox® preservative.

Add one volume of Wash Fluid Concentrate to 19 volumes of distilled or deionised water to give the required volume or dilute the entire contents of one bottle of Wash Fluid to a final volume of 2500 ml. Crystals may be observed in the Wash Fluid Concentrate but these crystals will dissolve when the Wash Fluid is diluted to working strength. When diluted, the Wash Fluid contains 0.01% Bronidox® preservative.

The Wash Fluid from this kit may be used interchangeably with the Glycine/Borate Wash Fluid from any other Murex kit.

Store the working strength Wash Fluid at 18 to 30°C in a closed vessel under which conditions it will retain activity for one month.

NOTE: The Wash Fluid may develop a yellow colour on storage. This will have no effect on the performance of the assay providing the Wash Fluid is fully aspirated from the wells.

NOTE: Although the Substrate Solution and Wash Fluid are interchangeable, they must not be used beyond the expiry date printed on the component labels.

WARNINGS AND PRECAUTIONS

IVD

The reagents are for *in vitro* diagnostic use only.

For professional use only.

Please refer to the manufacturer's safety data sheet and the product labelling for information on potentially hazardous components.

HEALTH AND SAFETY INFORMATION



CAUTION: This kit contains components of human origin.

The human sera used for manufacture have been screened and found reactive or non-reactive for analytes as shown in **Table 2** below.

Table 2

Component	Reactive for	Non-reactive for
Negative Control	N/A	HBsAg, and antibodies to HIV-1 and 2, HCV and HTLV I + II
Positive Control	HBsAg	Antibodies to HIV-1 and HIV-2, and HCV

All reactive serum used has been inactivated prior to use in reagent preparation. However, all material of human origin should be considered as potentially infectious and it is recommended that this kit and test specimens be handled using established good laboratory practice.

The Sample Diluent and Conjugate contain 0.05% ProClin® 300 which is classified per applicable European Economic Community (EEC) Directives as irritant (Xi). The following are the appropriate Risk (R) and Safety (S) phrases.

Xi



- | | |
|-----|---|
| R43 | May cause sensitisation by skin contact. |
| S24 | Avoid contact with skin. |
| S35 | This material and its container must be disposed of in a safe way. |
| S37 | Wear suitable gloves. |
| S46 | If swallowed, seek medical advice immediately and show this container or label. |

Information for European customers: For product not classified as dangerous per European Directive 1999/45/EC - Safety Data Sheet available for professional user on request.

- Potentially contaminated materials should be disposed of safely according to local requirement.
- Spillage of potentially infectious material should be removed immediately with absorbent paper tissue and the contaminated area swabbed with, for example, 1.0% sodium hypochlorite before work is continued³. Sodium hypochlorite should not be used on acid containing spills unless the spill area is first wiped dry. Materials used to clean spills, including gloves, should be disposed of as potentially biohazardous waste. Do not autoclave materials containing sodium hypochlorite.
- Neutralised acids and other liquid waste should be decontaminated by adding a sufficient volume of sodium hypochlorite to obtain a final concentration of at least 1.0%. A 30 minute exposure to 1.0% sodium hypochlorite may be necessary to ensure effective decontamination.
- Do not pipette by mouth. Wear disposable gloves and eye protection while handling specimens and performing the assay. Wash hands thoroughly when finished.
- The following reagents contain low concentrations of harmful substances.
 - The Conjugate and Sample Diluent contain detergents.
- Sulphuric acid required for the Stop Solution and hydrochloric acid used for washing glassware are corrosive and should be handled with appropriate care. If either come into contact with the skin or eyes, wash thoroughly with water.
- If any of the reagents come into contact with the skin or eyes wash the area extensively with water.

ANALYTICAL PRECAUTIONS

1. Do not use the reagents beyond the stated expiry date. Microbiological contamination of reagents must be avoided as this may reduce the life of the product and cause erroneous results.
2. Do not modify the **Test Procedure** or substitute reagents from other manufacturers or other lots unless the reagent is stipulated as interchangeable. Do not reduce any of the recommended incubation times.
3. Allow all reagents and samples to come to 18 to 30°C before use. Immediately after use return all reagents to the recommended storage temperature.
4. Any glassware to be used with the reagents should be thoroughly washed with 2M hydrochloric acid and then rinsed with distilled water or high quality deionised water.
5. Avoid the use of self-defrosting freezers for the storage of reagents and samples.
6. Do not expose reagents to strong light or hypochlorite fumes during storage or during incubation steps.
7. Do not allow wells to become dry during the assay procedure.
8. Do not cross-contaminate reagents. Dedicate a pipette for use with the Substrate Solution of Murex assays. A pipette should also be dedicated for use with the Conjugate.
9. Do not touch or splash the rim of the well with Conjugate. Do not blow out from micropipettes; reverse pipetting is recommended wherever possible.
10. Ensure that the bottom of the plate is clean and dry and that no bubbles are present on the surface of the liquid before reading the plate.
11. Do not contaminate microwells with the dust from disposable gloves.
12. When using fully automated microplate processors
 - i) It is not necessary to use plate lids and to tap dry the wells.
 - ii) Do not allow system fluids from fully automated microplate processors to contaminate samples or reagents.
 - iii) The possibility of cross contamination between assays needs to be excluded when validating assays on fully automated processors
13. Ensure the assay is run within the temperature limits defined in the assay protocol.
14. Do not use CO₂ incubators.
15. Do not store the Stop Solution in a shallow dish or return it to a stock bottle after use.
16. The possibility of cross contamination between assays needs to be excluded when validating assay protocols on instrumentation.

SPECIMEN COLLECTION, TRANSPORT AND STORAGE

SPECIMEN COLLECTION

Serum, EDTA plasma or citrate plasma samples may be used. Blood collected by venepuncture should be allowed to clot naturally. Ensure that the serum samples are fully clotted. Remove any visible particulate matter from the sample by centrifugation. If samples are prepared using liquid anti-coagulants e.g. citrate plasma, the dilution effect should be considered.

SPECIMEN TRANSPORT AND STORAGE

Store samples at 2 to 8°C. Samples not required for assay within 72 hours should be removed from the clot or cell pellet and stored frozen (-15°C or colder). Avoid multiple freeze-thaw cycles. After thawing, ensure samples are thoroughly mixed before testing.

PROCEDURE

MATERIALS REQUIRED BUT NOT PROVIDED

1. **Stop Solution (0.5M to 2M Sulphuric Acid)**. e.g. add between 3 ml (for 0.5M) and 11 ml (for 2.0M) of analytical grade concentrated sulphuric acid (18.0M) to about 80 ml of distilled or deionised water and then make up to 100 ml with more water. Alternatively, the following reagent can be used; 1N Sulphuric Acid (Code N0164).
2. **Freshly distilled or high quality deionised water** is required for dilution of Wash Fluid, for preparation of the Stop Solution and for use in conjunction with automated washers.
3. **Micropipettes and Multichannel micropipettes** of appropriate volume.
4. **Incubator** capable of maintaining the temperature limits defined in the assay protocol.
5. **Moulded Heating Block** (Code 5F09-02). For use in laboratory incubators. The moulded heating block should ideally be kept in the incubator used. If this is not possible it must be placed in the incubator at least four hours before beginning the assay.
6. **Instrumentation**
 - a) Automated microplate strip washer.
 - b) Microplate reader.or
 - c) Fully automated microplate processor.All instruments must be validated before use.
Please contact your representative for details of recommended systems, software protocols for instrumentation and validation procedures.
7. **Disposable Reagent Troughs**. (Code 5F24-01).
8. **Sodium hypochlorite** for decontamination. (Refer to **Health and Safety Information**).
9. **Sodium hydroxide solution (0.1M)** (For instrument decontamination).

TEST PROCEDURE

Please read '**Analytical Precautions**' carefully before performing the test.

Addition of the various components of the assay to the wells may be confirmed visually by examining the plate for the following colours:

Sample Diluent is green/brown in colour. On addition of the Sample or Control the colour will change to blue/green. The colour change will vary from sample to sample but some change should always be visible.

Conjugate is red in colour.

Substrate Solution is initially pink with any reactive wells becoming purple. On addition of Stop Solution the purple colour of the reactives will change to orange, whilst the negatives remain pink.

The addition of sample or reagent can be confirmed using a microplate reader as follows: Sample Diluent plus Sample read at 570 or 620 nm with a reference at 690 nm, Conjugate at 490 nm with a reference at 690 nm, Substrate Solution at 490 nm (no reference).

SEMI AUTOMATED PROCESSING

Step 1	Prepare Substrate Solution and Wash Fluid .	
Step 2	Use only the number of wells required for the test.	
Step 3	Add 25 µl of Sample Diluent to each well.	25 µl
Step 4	Add 75 µl of Samples or Controls to the wells. To each plate add 75 µl of the Negative Control to wells A1 and B1 and 75 µl of Positive Control into well C1. Add the Controls to the designated wells after dispensing the samples.	75 µl
Step 5	Cover the plate with a lid and incubate for 60 minutes at 37°C ±1°C.	60 mins
Step 6	Add 50 µl of Conjugate to each well.	50 µl
Step 7	Shake the plate using a plate shaker for 10 seconds or manually agitate by gently tapping the sides for 10 seconds.	10 secs
Step 8	Cover the plate with the lid and incubate for 30 minutes at 37°C ±1°C.	30 mins
Step 9	At the end of the incubation time wash the plate 5 times as described under Wash Procedures . After washing is completed invert the plate and tap out any residual Wash Fluid onto absorbent paper.	5 washes
Step 10	Immediately after washing the plate, add 100 µl of Substrate Solution to each well.	100 µl
Step 11	Cover the plate with a lid and incubate for 30 minutes at 37°C ±1°C while colour develops. A purple colour should develop in wells containing reactive samples.	30 mins
Step 12	Add 50 µl of Stop Solution to each well.	50 µl
Step 13	Within 15 minutes read the absorbance of each well at 450 nm using 620 nm to 690 nm as the reference wavelength if available. Blank the instrument on air (no plate in the carriage).	A_{450/Ref}

WASH PROCEDURES

Protocols for recommended washers and procedures for verifying washers and analysers can be obtained from your representative. The following protocol is recommended:

a) Protocol for automated microplate stripwasher

Perform 5 wash cycles using working strength Wash Fluid. Ensure, where possible, that:

- Flow-through washing with a fill volume of 500 µl/well is used with instrumentation supplied by DiaSorin. When using other instrumentation for which this is not possible, ensure that the well is completely filled.
- The dispense height is set to completely fill the well with a slight positive meniscus, without causing an overflow.
- The time taken to complete one aspirate/wash/soak cycle is approximately 30 seconds.
- Ensure that no liquid is left in the well (by use of a double aspirate step in the final cycle where possible).
- After washing is completed, invert the plate and tap out any residual Wash Fluid onto absorbent paper.

NOTE: Do not allow the wells to become dry during the assay procedure.

Washers must be rinsed with distilled water at the end of the test to avoid blockage and corrosion.

FULLY AUTOMATED MICROPLATE PROCESSORS.

Contact your representative for details of currently available validated protocols. For instrumentation without established validated protocols, the following guidelines are recommended:

- Do not programme times shorter than specified in the procedure.
- For each incubation at 37°C, programmed times may be increased by up to 20%.
- Wells containing Sample Diluent may be left for up to 60 minutes at 18 to 30°C prior to addition of sample and controls and for up to 60 minutes after the addition of sample and controls before starting step 5.
- Ensure all '**Analytical Precautions**' are followed.
Protocols written following these guidelines must be fully validated prior to use according to local procedures.

RESULTS

CALCULATION OF RESULTS

Each plate must be considered separately when calculating and interpreting results of the assay.

Approved software may be used for calculation and interpretation of results.

Negative Control

Calculate the mean absorbance of the replicates of the Negative Control.

If one of the Negative Control wells has an absorbance more than 0.03 above the other discard the higher value.

Cut-off Value

Calculate the Cut-off Value by adding 0.05 to the mean of the Negative Control replicates.

Example

Negative Control absorbance: well 1 = 0.071, well 2 = 0.075

Mean Negative Control = (0.071 + 0.075)/2 = 0.073

Cut-off Value = 0.073 + 0.05 = 0.123

QUALITY CONTROL

Results of an assay are valid if the following criteria for the controls are met:

Negative Control

The mean $A_{450/Ref}$ of the Negative Control is less than 0.15 or the mean A_{450} of the Negative Control is less than 0.2.

Positive Control

The $A_{450/Ref}$ or A_{450} of the Positive Control is more than 0.8 above the mean $A_{450/Ref}$ or A_{450} of the Negative Control.

Assays which do not meet these criteria should be repeated.

In the unlikely event of the results repeatedly failing to meet either the Quality Control criteria or the expected performance of the test, please contact your representative.

INTERPRETATION OF RESULTS

Non Reactive Results

Samples giving an absorbance less than the Cut-off Value are considered non-reactive in Murex HBsAg Version 3.

Reactive Result

Samples giving an absorbance equal to or greater than the Cut-off Value are considered initially reactive in the assay (see **Limitations of the Procedure**). Such samples should be retested in duplicate using the original sample source. Samples that are reactive in at least one of the re-tests are presumed to contain HBsAg and should be confirmed by using the Murex HBsAg Confirmatory Version 3 kit (2G27-01) and tests for other HBV markers. Samples that are non-reactive in both wells on retest should be considered non-reactive.

SPECIFIC PERFORMANCE CHARACTERISTICS

The performance of Murex HBsAg Version 3 has been determined by testing samples from random blood donors, patients with acute and chronic hepatitis B infection, patients with mutant forms of hepatitis B infection and patients with diseases unrelated to hepatitis B infection.

In addition, its performance against the French A.F.S.S.A.P.S. panels and other commercially available seroconversion samples has been evaluated.

1. Donor Samples

The Murex HBsAg assay demonstrated a specificity of $\geq 99.5\%$ in a study where a total of 12330 routine donor samples were screened with Murex HBsAg Version 3. In the study, 0.18% (22/12330) of samples were initially reactive and 0.03% (4/12330) were repeatedly reactive. None of the repeatedly reactive samples with Murex HBsAg version 3 and the alternative assays were confirmed as positive for the presence of hepatitis B surface antigen.

The specificity of Murex HBsAg Version 3 on presumed negative samples from donors is estimated to be 99.97% (12326/12330) with a 95% confidence interval of 99.92% (12320/12330) to 99.99% (12329/12330) by the binomial distribution.*

2. Clinical Samples

Samples from patients at various stages of hepatitis B infection and patients with conditions unrelated to hepatitis B were tested in three regional virus reference laboratories and at DiaSorin.

A total of 630 samples from patients suffering from acute and chronic hepatitis B infection were tested with Murex HBsAg version 3. All 630 samples were confirmed with an alternative immunoassay for HBsAg and found to be positive in both Assays.

A further six samples from patients infected with mutant forms of hepatitis B infection, confirmed by DNA sequencing, were also tested with Murex HBsAg version 3 and were all detected successfully.

In addition 998 potentially cross-reactive samples from patients with conditions unrelated to hepatitis B infection, including other acute viral infections, antenatal, lipaemic, icteric and haemolysed samples, were tested with Murex HBsAg Version 3. A total of 996 of these samples were non-reactive with Murex HBsAg Version 3. Of the two repeatedly reactive samples, one was false reactive and showed no other hepatitis markers, the remaining sample was anti-HBc positive.

3. Seroconversion Panels

A total of 22 commercially available HBV seroconversion panels were tested with Murex HBsAg Version 3. Comparison with two other commercially available microplate based immunoassays for the detection of hepatitis B surface antigen showed that Murex HBsAg Version 3 detected HBsAg six bleeds earlier in one panel, four bleeds earlier in one panel, two bleeds earlier in one panel, one bleed earlier in four panels and at the same bleed in the remaining 15 panels.

4. Assay Reproducibility

Ten replicates of each of five samples were tested on ten separate test occasions with two separate batches to ascertain the reproducibility of Murex HBsAg Version 3. The results of the study are summarised in Table 3 and Table 4.

*Representative performance data are shown: results obtained at individual laboratories and with different populations may vary.

Table 3
Murex HBsAg Version 3 - Assay Reproducibility (Kit 1)

Specimen	Number of Assays	Mean Absorbance/ Cut-off Value	Intra-assay %CV	Inter-assay %CV
1	10	3.36	7.6	9.2
2	10	1.29	8.2	9.5
3	10	4.44	10.1	11.5
4	10	0.68	9.3	10.8
5	10	0.25	11.0	12.9

Table 4
Murex HBsAg Version 3 - Assay Reproducibility (Kit 2)

Specimen	Number of Assays	Mean Absorbance/ Cut-Off Value	Intra-assay %CV	Inter-assay %CV
1	10	3.19	6.8	7.9
2	10	1.19	7.3	9.1
3	10	3.73	7.7	8.5
4	10	0.55	9.2	12.6
5	10	0.18	7.5	8.3

LIMITATIONS OF THE PROCEDURE

- The **Test Procedure** and **Interpretation of Results** must be followed.
- This test has only been evaluated for use with individual (unpooled) serum, EDTA plasma, or citrate plasma samples.
- A negative result with an antigen detection test does not preclude the possibility of infection.
- Non-repeatable reactive results may be obtained with any EIA procedure.
- The most common sources of error are:
 - Imprecise delivery of Sample, Conjugate or Substrate into the wells.
 - Contamination of Substrate with Conjugate.
 - Contamination with conjugates from other assays
 - Blocked or partially blocked washer probes.
 - Insufficient aspiration leaving a small volume of Wash Fluid in the wells.
 - Failure to ensure that the bottom surface of the wells is clean and dry, and that no air bubbles are present on the surface of the liquid in the wells before a plate is read.
 - Failure to read at the correct wavelength or use of an incorrect reference wavelength.
- The use of highly haemolysed samples, incompletely clotted sera, plasma samples containing fibrin or samples with microbial contamination may give rise to erroneous results.
- This test has not been evaluated for use with samples from cadavers.

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