Murex HIV Ag/Ab Combination is an enzyme immunoassay for the simultaneous qualitative detection of Human Immunodeficiency Virus (HIV) p24 antigen and antibodies to HIV type 1 (HIV-1 group O) and HIV type 2 (HIV-2) in human serum or plasma. This kit is intended as an aid in the diagnosis of HIV-1 and/or HIV-2 infection. Murex HIV Ag/Ab Combination is intended for manual use with an automated microplate washer and reader, and for use with fully automated microplate instrumentation using a validated protocol. Results from Murex HIV Ag/Ab Combination cannot be used to distinguish between the presence of HIV-1 p24 antigen, HIV-1 antibody, or HIV-2 antibody in a specimen.

Murex HIV Ag/Ab Combination is intended for screening individual human donors (blood or plasma) for the presence of HIV-1 p24 antigen and antibodies to HIV-1 (including subtype O) and HIV-2, and as an aid to diagnosis.

Murex HIV Ag/Ab Combination is based on microwells coated with synthetic peptide representing immunodominant regions of HIV-1 (O) and HIV-2, recombinant protein derived from the envelope regions of HIV-1 and HIV-2 and HIV pol protein, together with monoclonal antibodies raised against p24 of HIV-1. The Conjugate is a mixture of the same antigen epitopes, and different monoclonal antibodies, also raised against p24, all labelled with horseradish peroxidase.

Test and control specimens are incubated in the wells and reactive HIV-1 p24 antigen and/or antibodies to HIV-1/2 in the test or control specimens sera bind to the antibodies and/or antigens on the microwell; sample and any excess antibodies or antigen are then washed away. In a subsequent step, Conjugate is added which in turn binds to any reactive HIV-1 p24 antigen and/or specific antibodies to HIV-1/2 already bound to the reagents on the well. Specimen not containing HIV-1 p24 antigen or specific antibodies to HIV-1/2 will not cause the Conjugate to bind to the well. Unbound Conjugate is washed away and a
solution containing 3,3',5,5'- tetramethylbenzidine (TMB) and hydrogen peroxide is added to the wells. Wells with bound Conjugate develop a blue green colour which is converted to an orange colour which may be read at 450nm after the reaction has been stopped with sulphuric acid.

Specimens giving an absorbance equal to or greater than the Cut-off value are considered initially reactive in the assay (see Limitations of the Procedure).

Unless local procedures state otherwise, such specimens must be repeated in duplicate using the original source specimen. Specimens that are reactive in at least one of the duplicate repeat tests are considered repeatedly reactive in Murex HIV Ag/Ab Combination and are presumed to contain HIV-1 p24 antigen and/or antibodies to HIV-1 or HIV-2. Such specimens must be further investigated and the results of this assay considered with any other clinical and supplemental testing. Specimens that are non-reactive in both wells on repeat testing are considered non-reactive for HIV-1 p24 antigen and antibodies to HIV-1/2.

The test kit contains:

<table>
<thead>
<tr>
<th></th>
<th>96 tests (product code 7G79-09)</th>
<th>480 tests (product code 7G79-11)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Coated Wells</strong></td>
<td>One plate</td>
<td>Five plates</td>
</tr>
<tr>
<td>Coated Wells</td>
<td>96 microwells coated with HIV antigens and monoclonal antibodies</td>
<td></td>
</tr>
<tr>
<td><strong>Sample Diluent</strong></td>
<td>1 bottle of 8 ml</td>
<td>1 bottle of 18 ml</td>
</tr>
<tr>
<td>Sample Diluent</td>
<td>Green/brown buffered solution containing bovine and murine protein, detergent and saponin. Contains 0.05% ProClin® 300 preservative.</td>
<td></td>
</tr>
<tr>
<td><strong>Conjugate</strong></td>
<td>1 bottle of 1.1 ml</td>
<td>3 bottles of 1.1 ml</td>
</tr>
<tr>
<td>Conjugate</td>
<td>HIV antigens and monoclonal antibodies conjugated to horseradish peroxidase and freeze dried. When reconstituted each bottle is sufficient for up to two plates.</td>
<td></td>
</tr>
<tr>
<td><strong>Conjugate Diluent</strong></td>
<td>1 bottle of 22 ml</td>
<td>3 bottles of 22 ml</td>
</tr>
<tr>
<td>Conjugate Diluent</td>
<td>Yellow buffered solution consisting bovine protein, saponin and detergent. Sufficient to reconstitute one bottle of Conjugate</td>
<td></td>
</tr>
</tbody>
</table>
| **Anti-HIV-1 Positive Control**  
| Inactivated human serum in a buffer containing bovine protein. | 1 bottle of 1.7 ml | 1 bottle of 1.7 ml |
| **Anti-HIV-2 Positive Control**  
| Inactivated human serum in a buffer containing bovine protein. | 1 bottle of 1.7 ml | 1 bottle of 1.7 ml |
| **HIV-1 p24 Positive Control**  
| HIV-1 p24 (recombinant antigen) in a buffer containing bovine protein. | 1 bottle of 1.7 ml | 1 bottle of 1.7 ml |
| **Negative Control**  
| Normal human serum in a buffer containing bovine protein. | 2 bottles of 2.5 ml | 2 bottles of 2.5 ml |
| **Substrate Diluent**  
| Colourless solution of tri-sodium citrate and hydrogen peroxide. | 1 bottle of 35 ml | 1 bottle of 35 ml |
| **Substrate Concentrate**  
| 3,3',5,5'-tetramethylbenzidine (TMB) and stabilizers in an orange solution. | 1 bottle of 35 ml | 1 bottle of 35 ml |
| **Wash Fluid**  
| 20 times working strength Glycine Borate Wash Fluid. | 1 bottle of 125 ml | 2 bottles of 125 ml |

**Note:** a copy of the instructions for use is not part of the test kit components, and must be requested separately from the manufacturer, or the local distributor.

**Storage:**
2 to 8 °C (for all components, under which condition they will retain activity until the expiry date of the kit)

**Shelf-life:**
12 months.
Summary of prequalification status for Murex HIV Ag/Ab Combination

<table>
<thead>
<tr>
<th>Initial acceptance</th>
<th>Date</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>PQ status amended</td>
<td>17 November 2016</td>
<td>listed</td>
</tr>
<tr>
<td>Status on PQ list</td>
<td>30 March 2015</td>
<td>listed</td>
</tr>
<tr>
<td>Dossier assessment</td>
<td>22 July 2014</td>
<td>MR</td>
</tr>
<tr>
<td>Inspection status</td>
<td>12 August 2014</td>
<td>MR</td>
</tr>
<tr>
<td>Laboratory evaluation</td>
<td>18 February 2014</td>
<td>MR</td>
</tr>
</tbody>
</table>

MR: Meets Requirements
N/A: Not Applicable

Murex HIV Ag/Ab Combination was accepted for the WHO list of prequalified in vitro diagnostics on the basis of data submitted and publicly available information.

Background information

DiaSorin S.p.A UK Branch submitted an application for prequalification of Murex HIV Ag/Ab Combination. Based on the established prioritization criteria, Murex HIV Ag/Ab Combination was given priority for prequalification.

Product dossier assessment

DiaSorin S.p.A UK Branch submitted a product dossier for Murex HIV Ag/Ab Combination as per the Instructions for compilation of a product dossier (PQDx_018 v3). The information submitted in the product dossier was reviewed by WHO staff and external experts (assessors) appointed by WHO in accordance with the internal report on the screening and assessment of a product dossier (PQDx_009 v2). Based on the product dossier screening and assessment findings, a recommendation was made to accept the product dossier for Murex HIV Ag/Ab Combination for prequalification.

Commitments for prequalification:
1. Updated instructions for use.

Manufacturing site inspection

A comprehensive inspection was performed at the site of manufacture (Central Road, Dartford, Kent, DA1 5LR, UK) and the site of warehousing (Via Crescentino, snc, 13040 Saluggia, Italy) of Murex HIV Ag/Ab Combination in February 2014 as per the Information for manufacturers on prequalification inspection procedures for the sites of manufacture of diagnostics (PQDx_014 v3). The inspection found that the manufacturer had an acceptable quality management system and good manufacturing practices in place that ensured the consistent manufacture of a product of good quality. The manufacturer's
responses to the nonconformities found at the time of the inspection were accepted and successfully closed on 11 August 2014.

Laboratory evaluation
Murex HIV Ag/Ab Combination was evaluated by WHO at the Institute of Tropical Medicine, Antwerp, Belgium – a WHO Collaborating Centre for HIV/AIDS Diagnostics and Laboratory Support. The laboratory evaluation was conducted according to the “WHO protocol for the laboratory evaluation of HIV serology assays” (PQDx_030 v1.0), and drew the following conclusions:

Murex HIV Ag/Ab Combination is a qualitative 4th generation sandwich enzyme immunoassay intended to screen individual human donors for the presence of HIV p24 antigen and antibodies to HIV-1, including group O, and HIV-2 or as an aid to the diagnosis of HIV infection. A volume of 100 µl of specimen is needed to perform the assay. This type of assay requires laboratory equipment and cannot be performed in laboratories with limited facilities. Reading of the results must be performed with a spectrophotometer.

In this limited performance evaluation on a panel of 1119 specimens, we found an initial sensitivity (95% CI) of 100% (99.2 - 100%) and an initial specificity (95% CI) of 99.4% (98.4 - 99.8%) compared to the reference results. The final sensitivity (95% CI) was 100% (99.2 - 100%) and the final specificity (95% CI) was 99.7% (98.9 - 100%) compared to the reference results. Lot to lot variation observed was within the acceptance range.

For eight seroconversion panels, Murex HIV Ag/Ab Combination detected on average 1.125 specimens earlier than the benchmark assay (Enzygnost Anti-HIV 1/2 Plus [Siemens Healthcare Diagnostics]) and on average 0.5 specimens earlier than Vironostika HIV Ag/Ab (bioMérieux) EIA.

For the mixed titer panel, Murex HIV Ag/Ab Combination correctly classified all specimens. For the HIV-1 p24 antigen panel, Murex HIV Ag/Ab Combination classified all but one of HIV-1 antigen positive/anti-HIV negative specimens. For the HIV culture supernatant panel, Murex HIV Ag/Ab Combination identified all HIV-1 and HIV-2 subtypes.

For the 1st International Reference Panel for anti-HIV [NIBSC code 02/210], Murex HIV Ag/Ab Combination detected all subtypes tested (HIV-1 A, HIV-1 B, HIV-C, HIV-1 CRF01_AE, HIV-1 O and HIV-2). For the HIV-1 p24 antigen standard [NIBSC code 90/636], Murex HIV Ag/Ab Combination detected to 1.56 international units. In contrast, Vironostika HIV Ag/Ab (bioMérieux) detected to 12.5 international units.

In the study, 0% of the results were recorded as indeterminate and the invalid rate was 0%.

Change notification
In 2016, DiaSorin S.p.A UK Branch submitted a change notification related to changes in labelling. This change notification was assessed and product was found to meet WHO prequalification requirements.
Labelling

1. Labels
2. Instructions for use
1. Labels

Murex HIV Ag/Ab Combination (7G79-09/11)

Murex HIV Ag/Ab Combination

GE41/42
22 ml

DiaSorin

CONJUGATE DIL LOT
2°C
1
P137
P062
P336
P301

3LA2DS41C

Murex HIV Ag/Ab Combination

GE41/42
1.1 ml

DiaSorin

CONJUGATE LOT
2°C
1
P062
P336
P301

2LA4DS41E

Murex HIV Ag/Ab Combination

GE41
2 ml

DiaSorin

SAMPLE DIL LOT
2°C
1
P062
P336
P301

6LA1DS41B

Murex HIV Ag/Ab Combination

GE42
18 ml

DiaSorin

SAMPLE DIL LOT
2°C
1
P062
P336
P301

9LA1DS42B

DiaSorin

SUBSTRATE CONC LOT
2°C
1
P062
P336
P301

1DA8ITMBJ

DiaSorin

SUBSTRATE DIL LOT
2°C
1
P062
P336
P301

1DA6SUBDG

DiaSorin

WASH FLUID LOT
2°C
1
P062
P336
P301

1DA9125MG

DiaSorin

WASH FLUID (2x conc)
2°C
1
P062
P336
P301

1DA9125MG

DiaSorin

WASH FLUID (3x conc)
2°C
1
P062
P336
P301

1DA9125MG
2. Instructions for use

Murex HIV Ag/Ab Combination

Enzyme immunoassay for improved detection of seroconversion to human immunodeficiency virus types 1 (HIV-1, HIV-1 group O) and detection of anti-HIV-2 antibodies

The assay is intended to screen individual human donors for the presence of HIV p24 antigen and antibodies to HIV-1, including group O, and HIV-2 or as an aid to the diagnosis of HIV infection.

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.

Key to symbols used

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>REF</td>
<td>List Number</td>
</tr>
<tr>
<td>LOT</td>
<td>Lot Number</td>
</tr>
<tr>
<td></td>
<td>Expiration Date</td>
</tr>
<tr>
<td></td>
<td>Manufacturer</td>
</tr>
<tr>
<td>IVD</td>
<td>In Vitro Diagnostic Medical Device</td>
</tr>
<tr>
<td></td>
<td>Store at 2-8°C</td>
</tr>
<tr>
<td></td>
<td>CAUTION: Consult accompanying documents</td>
</tr>
<tr>
<td></td>
<td>Consult instructions for use</td>
</tr>
</tbody>
</table>

See REAGENTS section for a full explanation of symbols used in reagent component naming.
INTENDED USE
Enzyme immunoassay for improved detection of seroconversion to human immunodeficiency virus types 1 (HIV-1, group O) and detection of anti-HIV-2 antibodies.

The assay is intended to screen individual human donors for the presence of HIV p24 antigen and antibodies to HIV-1, including group O, and HIV-2 to aid in the diagnosis of HIV infection.

SUMMARY AND EXPLANATION OF THE TEST
Two types of human immunodeficiency virus, HIV-1 and HIV-2, have been described and implicated as causative of the Acquired Immunodeficiency Syndrome (AIDS). Both are retroviruses which are transmitted by exposure to infected bodily fluids, primarily blood and genital secretions, and by transplacental passage. Infection by HIV-1 has been reported worldwide;

HIV-2 infection has been reported as occurring mainly in West Africa and some European countries.

The two types of virus show substantial antigenic cross-reactivity in their gag and pol proteins, but the envelope glycoproteins are less cross-reactive.

It is necessary for screening purposes to use epitopes from the envelope proteins of both viruses in addition to major cross reacting gag or pol proteins to ensure detection of antibodies against both types of virus at all stages following infection. Variants of HIV-1, classified together as group O, have been identified in samples from Cameroon and Equatorial Guinea. Group O is highly divergent from the originally known subtypes of HIV-1 (together classified as group M). Specific epitopes from the envelope region of this virus can be used to detect antibody to group O in infected individuals. Reliance on cross reactions to the known subtypes of HIV is not satisfactory.

The earliest specific antibody response following infection by HIV may be of immunoglobulin M (IgM), followed by a response in immunoglobulin G (IgG). Maximum sensitivity for detection of anti-HIV seroconversion is achieved by assays which respond to both IgM and IgG whilst HIV core antigens is typically detectable during a short period prior to antibody seroconversion.

Murex HIV Ag/Ab Combination is designed to detect reactive HIV core antigen in addition to IgG, IgM and IgA to the envelope glycoproteins and the cross reacting pol proteins of HIV-1 and HIV-2. Consequently potentially infectious samples of serum, EDTA plasma or citrate plasma can be identified.

PRINCIPLE OF THE PROCEDURE
Murex HIV Ag/Ab Combination is based on microtiter plates coated with synthetic peptide representing immunodominant regions of HIV-1 (O) and HIV-2, recombinant protein derived from the envelope regions of HIV-1 and HIV-2 and pol protein together with monoclonal antibodies raised against p24 of HIV-1. The Conjugate is a mixture of the same antigen epitopes, and different monoclonal antibodies, also raised against p24, all labelled with horse-radish peroxidase.

Test specimens and control sera are incubated in the wells and reactive HIV core and/or antibodies to HIV in the sample or control sera bind to the antibodies and/or antigens on the microtiter sample and any excess antibodies are then washed away. In a subsequent step, Conjugate is added which in turn binds to any reactive HIV core and/or specific antibody already bound to the reagents on the well. Samples not containing reactive core antigen or specific antibody will not cause the Conjugate to bind to this wall.

Unbound Conjugate is washed away and a solution containing 3%, 5%, and 10% 3-3',5'-teramethybenzidine (TMB) and hydrogen peroxide is added to the wells. Wells with bound Conjugate develop a blue green colour which is converted to an orange colour which may be read at 450nm after the reaction has been stopped 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 (7G79-09) or five plates (7G79-11) of 96 microtiter coated with HIV antigens and monoclonal antibodies.

   Allow the wells to reach room temperature (18 to 30°C) before removal from the bag.

   Place unused wells in the storage bag provided and return to 2 to 8°C.

2. Sample Diluent
   - One bottle containing 8 ml (7G79-09) or 18 ml (7G79-11) of a green/brown buffer solution, bovine and murine protein, detergent and sorbitol. Contains 0.05% ProClin® 300 preservative.

3. Conjugate
   - One bottle (7G79-09) or three bottles (7G79-11) containing 11 ml of HIV antigens and monoclonal antibodies conjugated to horseradish peroxidase and freeze dried. When reconstituted each bottle is sufficient for up to two plates.

4. Conjugate Diluent
   - One bottle (7G79-09) or three bottles (7G79-11) containing 20 ml of yellow solution consisting of buffer, bovine protein, sorbitol and detergent, sufficient to reconstitute one bottle of Conjugate. Contains 0.1% ProClin® 300 preservative.

**RECONSTITUTION OF CONJUGATE**

- Tap the bottle of Conjugate gently on the bench to remove any material adhering to the rubber stopper. Pour the whole contents of the bottle of conjugate diluent into the bottle of conjugate, recap the latter and mix by gentle inversion. Allow to rehydrate for at least 50 minutes with occasional swirling. The reconstituted conjugate will be red in colour. Reconstituted conjugates may be returned to and pooled in the plastic conjugate diluent bottles if required.

After reconstitution the Conjugate may be stored at 2 to 8°C for up to four weeks.

**CONTROL**

5. Anti-HIV-4 Positive Control
   - One bottle containing 1.7 ml of inactivated human serum in a buffer containing bovine protein. Contains 0.05% ProClin® preservative.

6. Anti-HIV-2 Positive Control
   - One bottle containing 1.7 ml of inactivated human serum in a buffer containing bovine protein. Contains 0.05% ProClin® preservative.

7. HIV-1 p24 Positive Control
   - One bottle containing 1.7 ml of p24 (recombinant antigen) in a buffer containing bovine protein. Contains 0.05% ProClin® preservative.

**CONTROL**

8. Negative Control
   - Two bottles containing 2.8 ml of normal human serum diluted in a bovine protein buffer. Contains 0.05% ProClin® preservative.
9. Substrate Diluent
One bottle containing 36 ml of a colourless solution of tri-sodium citrate and hydrogen peroxide.

10. Substrate Concentrate
One bottle containing 35 ml of 3,3',5,5'-tetramethylbenzidine (TMB) and stabilizers in an orange solution.

Substrate Solution
To prepare the Substrate Solution add a volume of colourless Substrate Diluent to an equal volume of orange 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

<table>
<thead>
<tr>
<th>Volume of Substrate Concentrate and Substrate Diluent Required</th>
<th>Number of Wells</th>
<th>Number of Plates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>8 16 24 32 40</td>
<td>48 64 72 80 96</td>
</tr>
<tr>
<td></td>
<td>0.5 1.0 2.0 2.5 3.0 3.5 4.0 4.5 5.0 6.0</td>
<td>8 12 18 22</td>
</tr>
<tr>
<td>Substrate Concentrate (ml)</td>
<td>8 16 24 32 40</td>
<td>48 64 72 80 96</td>
</tr>
<tr>
<td></td>
<td>0.5 1.0 2.0 2.5 3.0 3.5 4.0 4.5 5.0 6.0</td>
<td>8 12 18 22</td>
</tr>
</tbody>
</table>

Additional reagent may be required for use with automated systems. Keep away from sunlight. The Substrate Solution should be pale yellow. If it is green 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 Murix kits which use orange coloured Substrate Concentrate. Ensure that the Substrate Solution is prepared from the Substrate Diluent and Substrate Concentrate provided together.

The prepared Substrate Solution is stable refrigerated (2 to 8°C) or at 15 to 30°C for up to two days but it must be discarded if crystals have formed.

11. Wash Fluid
One (7G79-09) or two (7G79-11) bottles containing 125 ml of 20 times working strength Glycine/Borate Wash Fluid. Contains 0.2% Bromex® preservative.

Add one volume of Wash Fluid Concentrate to 19 volumes of distilled or deionized 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% Bromex® preservative.

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

Store the working strength Wash Fluid at 18 to 20°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 provided 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.

Low levels of fibrin precipitate may be observed in the Kit Controls and product performance is not affected by this. This is a product of certain serum batches used to manufacture the controls.

### 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 analysis as shown in Table 2 below:

### Table 2

<table>
<thead>
<tr>
<th>Component</th>
<th>Reactive for</th>
<th>Non-reactive for</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative Control</td>
<td>HbsAg, antibodies to HBV, HIV-1 and HIV-2</td>
<td></td>
</tr>
<tr>
<td>Positive Control 1</td>
<td>antibodies to HIV-1</td>
<td>HbsAg</td>
</tr>
<tr>
<td>Positive Control 2</td>
<td>antibodies to HIV-2</td>
<td>HbsAg</td>
</tr>
</tbody>
</table>

Additionally human sera used for positive controls are also tested for antibodies to HBV and may be reactive.

All reactive sera 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.

Pursuant to EC Regulation 1272/2008 (CLP) hazardous reagents are classified and labeled as follows:

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Symbols</th>
<th>Hazard Statements</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONJUGATE</td>
<td>!</td>
<td>R17 May cause an allergic skin reaction.</td>
</tr>
<tr>
<td>SAMPLE</td>
<td>!</td>
<td></td>
</tr>
<tr>
<td>CONJUGATE</td>
<td>!</td>
<td></td>
</tr>
</tbody>
</table>

**Classification**: Skin sens. 1 H377

**Signal Word**: Warning

**Warning**: R35 May cause respiratory irritation. Eye irritation. Eye protection/face protection. P333+P341 Skin irritation or rash occur. Get medical advice/attention.

**Dangers**: Reaction mass: 5-Chloro-2-Methyl-4-isothiazolin-3-one [EC no. 247-505-7] and 2-Methyl-4-isothiazolin-3-one [EC no. 220-229-5] (3:1)

The reconstituted Conjugate contains 0.1% ProClin® 950 which is classified hazardous per EC Regulation 1272/2008.
<table>
<thead>
<tr>
<th>Rationale</th>
<th>SUBSTRATE</th>
<th>CONC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Classification</td>
<td>Eye Irrit. 2</td>
<td>H319</td>
</tr>
<tr>
<td>Signal word</td>
<td>Warning</td>
<td></td>
</tr>
<tr>
<td>Symbols</td>
<td>!</td>
<td></td>
</tr>
<tr>
<td>Phrases</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hazard Statements</td>
<td>G319 Causes serious eye irritation</td>
<td></td>
</tr>
<tr>
<td>Precautionary Statements</td>
<td>P264 Wash hands thoroughly after handling P308/318/33: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.</td>
<td></td>
</tr>
</tbody>
</table>

Pursuant to EC Regulation 1907/2006 (CLP) WASH FLUID is labeled as H319, safety data sheets available on www.discom.com

**1.** Potentially contaminated materials should be disposed of safely according to local requirements.

**2.** Spillage of potentially infectious material should be removed immediately with absorbent paper tissue and the contaminated area disinfected, for example, 10% sodium hypochlorite before work is continued. 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.

**3.** 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 10%. A 30 minute exposure to 10% sodium hypochlorite may be necessary to ensure effective decontamination.

**4.** Do not pipette by mouth. Wear disposable gloves and eye protection while handling specimens and performing the assay. Wash hands thoroughly when finished.

**5.** If any of the reagents come into contact with the skin or eyes wash the area extensively with water.

**6.** 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.

**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 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 Mumps assays. A pipette should also be dedicated for use with the Conjugate.

**9.** The Sample Diluent in this assay has the potential to cause false positive results in anti-HIV1/HIV2 surface antigen (anti-HBs) assays if reagent cross contamination occurs.

If running Mumps HV Ag/Ab Combination in conjunction with an anti-HBs assay on a fixed tip instrument ensure that the possibility of cross contamination is excluded during the validation process.

10. Do not touch or splash the rim of the wells with Conjugate. Do not blow out from micropipettes; reverse pipetting is recommended whenever possible.

11. 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.

12. Do not contaminate micro wells with dust from disposable gloves.

13. When using fully automated processors
   i) It is not necessary to use plate lids and top dry the wells.
   ii) Do not allow system fluids to contaminate samples or reagents.
   iii) The possibility of cross contamination between assays needs to be excluded when validating assays on fully automated processors.

14. Ensure the assay is run within the temperature limits defined in the assay protocol.

15. Do not use CO2 incubators.

16. Do not store the Stop Solution in a shallow dish or return it to a stock bottle after use.

17. 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. 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 Sulfuric Acid), e.g. add between 3.0 ml (for 0.5M) and 11 ml (for 2.0M) of analytical grade concentrated sulphuric acid (98%) to about 60 ml of distilled or deionised water and then make up to 100 ml with more water. Alternatively, the following reagents can be used: 1N Sulphuric Acid (Code N016A - 50 x 500 ml pack and N015hs - 1 x 1000 ml pack)

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 SF09-02). 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.

**Instrumentation**

a) Automated microplate stripwasher.

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.


8. Sodium hypochlorite for decontamination. (Refer to Health and Safety Information)

9. Sodium hydroxide solution (0.1M). (Refer to Analytical Precautions)
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 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. The addition of sample or control may be confirmed using a microplate reader at 570 nm or 620 nm with a reference of 690 nm.

Reconstituted Conjugate is red in colour. The addition of Conjugate may be confirmed using a microplate reader at 490 nm with a reference of 690 nm.

Substrate Solution is initially pale yellow with any reactive wells becoming blue green. On addition of Stop Solution the blue green colour of the reagents will change to orange whilst the negatives will change to pink. The addition of Substrate Solutions may be confirmed using a microplate reader at 450 nm (no reference).

SEMI-AUTOMATED PROCESSING

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Reconstitute and mix the Conjugate, prepare the Substrate Solution and Wash Fluid.</td>
</tr>
<tr>
<td>2.</td>
<td>Use only the number of wells required for the test. Avoid touching the tops or bottoms of the wells.</td>
</tr>
<tr>
<td>3.</td>
<td>Add 25 µl of Sample Diluent to each well.</td>
</tr>
<tr>
<td>4.</td>
<td>Add 100 µl of Samples or 100 µl Controls to the wells.</td>
</tr>
<tr>
<td>5.</td>
<td>For each plate use the first column of wells for the assay Controls. Add the Controls to the designated wells after dispensing the samples.</td>
</tr>
<tr>
<td>6.</td>
<td>Use of a white background will aid visualisation of sample addition.</td>
</tr>
<tr>
<td>7.</td>
<td>Cover the wells with the lid and incubate for 60 minutes at 37°C ± 1°C.</td>
</tr>
<tr>
<td>8.</td>
<td>Immediately after washing, add 100 µl of Stop Solution to each well.</td>
</tr>
<tr>
<td>9.</td>
<td>At the end of the incubation time wash the plate as described under Wash Procedures.</td>
</tr>
<tr>
<td>10.</td>
<td>Immediately after washing, add 100 µl of Stop Solution to each well.</td>
</tr>
<tr>
<td>11.</td>
<td>Cover the wells with a lid and incubate for 30 minutes at 37°C ± 1°C.</td>
</tr>
<tr>
<td>12.</td>
<td>Keep away from direct sunlight. A blue green colour should develop in wells containing reactive samples.</td>
</tr>
<tr>
<td>13.</td>
<td>Add 60 µl of Stop Solution (0.5 M to 2 M sulphuric acid) to each well.</td>
</tr>
<tr>
<td>14.</td>
<td>Within 15 minutes read the absorbance at 490 nm using 620 nm to 690 nm as the reference wavelength if available.</td>
</tr>
<tr>
<td>15.</td>
<td>Blank the instrument on air (no plate in the carriage).</td>
</tr>
</tbody>
</table>

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 stripwasher

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

(i) Flow-through washing with a 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.

(ii) The dispensing height is set to completely fill the well, with a slight positive meniscus, without causing an overflow.

(iii) The time taken to complete one aspirate/wash/soak cycle is approximately 30 seconds.

(iv) Ensure that no liquid is left in the well (by use of a double aspirate step in the final cycle where possible).

(v) 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 or deionised water at the end of the test to avoid blockage and contamination.

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

1. Do not programme times shorter than specified in the procedure.
2. For each incubation at 37°C, programmed times may be increased by up to 5 minutes.
3. Wells containing Sample Diluent may be left for up to 60 minutes at 18-20°C prior to the addition of Sample and for up to 60 minutes after the addition of samples or Controls before starting step 5 in the assay protocol.
4. 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 Negative Controls.

Example:

<table>
<thead>
<tr>
<th>Well</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.084</td>
</tr>
<tr>
<td>2</td>
<td>0.086</td>
</tr>
<tr>
<td>3</td>
<td>0.070</td>
</tr>
<tr>
<td>Total</td>
<td>0.340</td>
</tr>
</tbody>
</table>

Mean Negative Control = 0.240/3 = 0.080

If one of the Negative Control Wells has an absorbance more than 0.15 O.D. above the mean of all three, discard that value and calculate the new Negative Control mean from two remaining replicates.

Cut-off value

Calculate the Cut-off value by adding 0.160 to the mean of the Negative Control replicates (see above).

Mean Negative Control = 0.080
Cut-off value = 0.160 + 0.080 = 0.230
QUALITY CONTROL

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

Negative Control
The mean absorbance is less than 0.15.

Positive Controls
The absorbance of each of the Positive Controls is more than 0.8 above the mean absorbance 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 negative in the assay.

Reactive Results
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).

Unless local procedures state otherwise, such samples must be retested in duplicate using the original source. Samples that are reactive in at least one of the duplicate tests are considered repeatedly reactive in Murex HIV Ag/Ab Combination and must be retained to contain reactive HIV core antigen and/or antibodies to HIV-1 or HIV-2. Such samples must be further investigated and the results of this assay considered with any other clinical and/or assay information. Samples that are non-reactive in both wells on retest are considered non-reactive for HIV core antigen and HIV antibodies.

No sample addition
Absorbance values significantly higher than the Negative Control may be obtained in wells where the sample has been omitted but all the reagents have been added.

SPECIFIC PERFORMANCE CHARACTERISTICS

The performance of the Murex HIV Ag/Ab Combination has been determined by testing samples from random blood donors, patients with AIDS diagnosed according to CDC criteria, patients with AIDS-related complex (ARC), other patients with known antibody to HIV-1 (excluding group O), patients with confirmed HIV-2 infection and patients at risk of HIV infection or in other clinical categories. In addition, its performance on commercially available seroconversion panels has been evaluated.

Diagnostic Sensitivity
A total of 479 specimens from patients with confirmed HIV-1 infection were tested and found to be reactive with Murex HIV Ag/Ab Combination. The specimens were taken from patients at various stages of HIV infection and included 24 specimens from patients with HIV-1 subtype O infection and a further 19 specimens from patients infected with HIV-1 subtypes other than subtype B.

In addition, a total of 100 specimens from patients with confirmed HIV-2 infection were also tested with Murex HIV Ag/Ab Combination and found to be reactive.

The diagnostic sensitivity of the Murex HIV Ag/Ab Combination on this population of specimens is therefore estimated to be 100% (49 of 49) with a lower 95% confidence limit of 99.38% (69 of 69) by the binomial distribution.

A total of 26 commercial HIV-1 seroconversion panels were tested with Murex HIV Ag/Ab Combination. Using the presence of both core (p24) and an envelope (gp120/160) band on Western blot as the reference criteria, Murex HIV Ag/Ab Combination detected antibody to HIV earlier or in the same sample as Western blot in all of the panels.

Diagnostic Specificity
The Murex HIV Ag/Ab Combination assay demonstrated a specificity of ≥99.9%. In a study where specimens from a European blood donor population were tested. A total of 9,230 routine donor plasma specimens were screened with Murex HIV Ag/Ab Combination at three European blood transfusion centres. The results are summarised in Table 5. In the study, 89.77% (8,269/9,230) of specimens were non-reactive and 0.23% (21/9,230) were repeatedly reactive. One of the repeatedly reactive specimens was weakly positive with the Murex HIV Antigen mAb (82/77). None of the remaining 20 specimens were confirmed as positive for the presence of HIV-1 antigen or antibody to HIV-1 or HIV-2.

The specificity of Murex HIV Ag/Ab Combination on presumed negative European blood donors is estimated to be ≥99.7% (9,269/9,289) with 95% confidence limits of 99.67% (9,258/9,289) to 99.8% (9,271/9,289) by the binomial distribution.*

A total of 267 specimens from patients with conditions unrelated to HIV infection were also tested with Murex HIV Ag/Ab Combination. These included specimens from pregnant women and patients suffering with autoimmune disease and other acute viral infections. A total of 5 specimens were reactive with Murex HIV Ag/Ab, four were reactive with two other commercially available screening assays. In Western blot studies four produced indeterminate results and one was reactive. In addition, 18 lipemic, icteric and haemolyzed specimens were also tested and found to be non-reactive.

The overall diagnostic specificity of Murex HIV Ag/Ab Combination on confirmed negative specimens during this performance evaluation is estimated to be ≥99.7% (9,269/9,289) with 95% confidence limits of 99.67% (9,258/9,289) to 99.8% (9,271/9,289) by the binomial distribution.*

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

Assay Reproducibility
The reproducibility of Murex HIV Ag/Ab Combination was assessed by testing two of the assay controls and four quality assurance panel members as ten replicates on four separate occasions. The results from the testing are summarised in Table 4.

Table 3

<table>
<thead>
<tr>
<th>Reactivity of Murex HIV Ag/Ab Combination with presumed negative specimens from routine European blood donors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Centre</td>
</tr>
<tr>
<td>--------</td>
</tr>
<tr>
<td>A</td>
</tr>
<tr>
<td>B</td>
</tr>
<tr>
<td>C</td>
</tr>
<tr>
<td>TOTAL</td>
</tr>
</tbody>
</table>

*Includes one specimen which was weakly positive in Murex HIV Antigen mAb (82/77).

Table 4

<table>
<thead>
<tr>
<th>Murex HIV Ag/Ab Combination - Assay Reproducibility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specimen</td>
</tr>
<tr>
<td>----------</td>
</tr>
<tr>
<td>Negative Control</td>
</tr>
<tr>
<td>HIV-1 Positive Control</td>
</tr>
<tr>
<td>QA01</td>
</tr>
<tr>
<td>QA02</td>
</tr>
<tr>
<td>QA03</td>
</tr>
<tr>
<td>QA04</td>
</tr>
</tbody>
</table>

Sensitivity on AFSSAPS HIV Ag Standard

Sensitivity of Murex HIV Ag/Ab Combination on the AFSSAPS HIV Ag standard was determined at three testing centres.

Table 5

<table>
<thead>
<tr>
<th>Sensitivity on AFSSAPS HIV Ag standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>Centre</td>
</tr>
<tr>
<td>-------</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>Mean</td>
</tr>
</tbody>
</table>

The data shown in Table 5 was obtained during this testing but may not be exactly reproducible on other testing occasions.
LIMITATIONS OF THE PROCEDURE
1. The Test Procedure and Interpretation of Results must be followed.
2. This test has only been evaluated for use with individual (unpooled) serum, EDTA plasma or citrate plasma samples.
3. A negative result with an antigen/antibody detection test does not preclude the possibility of infection with HIV.
4. A positive result with Murex HIV Ag/Ab Combination should be confirmed by at least one other test.
5. Non-repeatable reactive results may be obtained with any RIA procedure.

The most common sources of error are:
   a) Imprecise delivery of Sample, Conjugate or Substrate into the wells.
   b) Contamination of Substrate with Conjugate.
   c) Contamination of conjugates from other assays.
   d) Blocked or partially blocked washer probe.
   e) Insufficient aspiration leaving a small volume of Wash Fluid in the wells.
   f) 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.
   g) Failure to read at the correct wavelength (450 nm) or use of an incorrect reference wavelength (not 620 nm to 690 nm).
6. The use of highly haemolysed samples, incompletely clotted sera, plasma samples containing fibrin or samples with microbial contamination may give rise to erroneous results.
7. This test has not been evaluated for use with samples from cadavers.

BIBLIOGRAPHY

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