DRG® HIV1+2 Ab (EIA-5217)

As of 24 May 2011 rm (Vers. 1.0) **USA: RUO**

Please use only the valid version of the package insert provided with the kit.

1 INTENDED USE

This ELISA kit is a Enzyme Immunoassay for the detection of circulating antibodies to Human Immunodeficiency Virus Type 1 (HIV-1) and/or Human Immunodeficiency Virus Type 2 (HIV-2) in human serum or plasma and is indicated as a screening test for serum or plasma and as an aid in the diagnosis of potential infection with HIV-1 and/or HIV-2.

2 SUMMARY

Human Immunodeficiency Virus Type (HIV-1) has been isolated from patients with AIDS and AIDS-related complex (ARC). HIV-1 was thought to be the sole causative agent of these syndromes until 1986, when a second type of Human Immunodeficiency Virus (HIV-2) was isolated and also reported to cause AIDS. Since the initial discovery, more than 600 cases of HIV-2 infection have been documented worldwide, with over 40 cases of AIDS related to HIV-2.

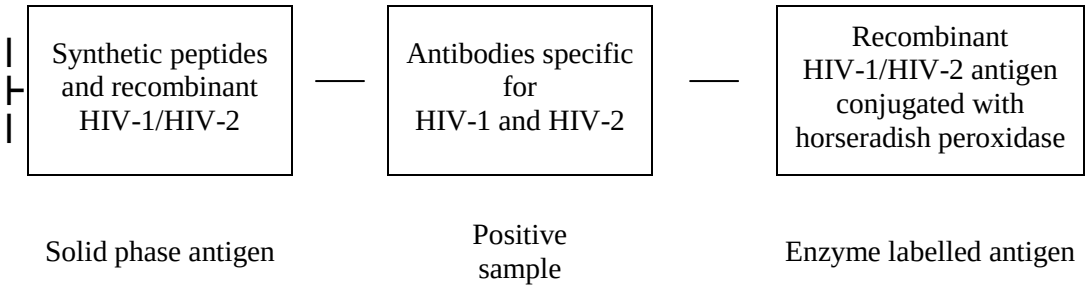
Both viruses have the same morphology and lymphotropism, and the modes of transmission appear to be identical. In addition, HIV-1 and HIV-2 genomes exhibit about 60% homology in conserved genes such as *gag* and *pol*. Serologic studies have also shown that the core proteins of HIV-1 and HIV-2 display frequent cross-reactivity whereas the envelope proteins are more type-specific.

Despite this immunologic cross-reactivity, detection of antibodies to HIV-2 with any of the licensed HIV-1 enzyme immunoassays is highly variable. This HIV-1/HIV-2 ELISA was developed to detect antibodies to HIV-1 and /or HIV-2, for blood screening and diagnostic purposes.

Any specimen that reacts in an initial test with the HIV-1/HIV-2 ELISA must be retested in duplicate with the HIV 1+2 Ab ELISA. Repeatably reactive specimens may contain antibodies to either HIV-1 or HIV-2. Therefore, additional, more specific or supplemental tests for antibodies to both HIV-1 and HIV-2 such as immunoblot, immunofluorescence, radioimmuno-precipitation must be performed to verify presence of antibodies to HIV.

3 PRINCIPLE OF THE ASSAY

The test is an enzyme-immunoassay based on a ‘sandwich’ principle.



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The HIV-1/HIV-2 EIA utilizes a detection system where microplate wells are coated with synthetic peptides and recombinant antigen corresponding to a highly antigenic segment of HIV-1/HIV-2 envelope and core proteins. Serum or plasma specimens, controls are added to the wells. During incubation, antibodies specific for HIV-1 and HIV-2 present in the specimen will bind to the peptides and recombinant antigen fixed onto the microplate wells. The wells are washed to remove unbound materials, and recombinant antigen conjugate will bind to the antigen-antibody complex and excess unbound enzyme conjugates are again removed by washing. The enzyme substrate, tetramethylbenzidine (TMB), is added upon incubation the substrate will be hydrolyzed by the bound enzyme and a blue or blue-green colour develops in wells containing HIV-1 and/or HIV-2 specific antibodies. The enzyme reaction is stopped by the addition of sulphuric acid. The intensity of colour developed is read spectrophotometrically at 450 nm and is proportional to the amount of antibodies present in the specimen.

4 KITS CONTENTS

1. **Coated Microplate**: 1 plate (96 tests) Twelve 8-well strips per plate.
   Each microplate well contains fixed HIV-1/HIV-2 specific synthetic peptides and recombinant antigen.
2. **Negative Control**: 1 vial of 1 ml.
   Normal human serum non-reactive for HBsAg and antibodies to HCV, HIV-1 and HIV-2. Contains sodium azide as preservative.
3. **Positive Control**: 1 vial of 1 ml.
   Inactivated human serum with high titer antibodies to HIV-1 and non-reactive for HBsAg and for HCV. Contains sodium azide as preservative.
4. **Conjugate:** 1 bottle of 12 ml.
   Phosphate buffered saline with Tween-20 containing normal goat serum, protein stabilizer and recombinant gp120, gp41, gp36 antigen peroxidase (horseradish) conjugate. Uses thimerosal as preservative.
5. **Wash Solution** Concentrate (25x): 1 bottle of 80 ml.
6. **Substrate Buffer (Chromogen A)**: 1 bottle of 8 ml, contains hydrogen peroxide.
7. **TMB (Chromogen B):** 1 bottle of 8 ml,
   citric buffer containing tetramethylbenzidine (TMB) and dimethylsulfoxide (DMSO).
8. **Stopping Solution**: 1 bottle of 7 ml,
   2M sulphuric acid solution.
9. **Plate Covers**: 4 pieces,
   Plastic covers for microplate during incubation.
10. **Plastic Pouch and Desiccant**:
    For unused strips.
11. **Instruction Manual**: 1 copy

***Note**: Store the kit at 2-8°C. Bring all reagents except HIV-1/HIV-2 EIA Conjugate to room temperature before use. Remove the Conjugate from 2-8°C immediately before use. Return to 2-8°C storage immediately after use.*

5 MATERIALS REQUIRED BUT NOT PROVIDED

Distilled water

Manual or automatic pipettors
Disposable pipette tips.
Timer
Microplate mixer
Incubator (37°C)
An automatic microplate washer (strongly recommended)
Microplate reader (equipped with a 450 nm and 630 nm filter)
Gloves

6 PRECAUTIONS FOR USERS

Do not use the kit after the expiration date.
Do not substitute reagents from one kit lot to another.
Do not pipette by mouth.
Use only reagent grade quality, deionised or distilled water to dilute reagents.
Do not expose substrate and TMB to strong light.
Avoid contact of TMB and sulphuric acid with any oxidizing agent or metal.
Avoid repeatedly opening and closing the incubator during incubation steps.

7 SAFETY PRECAUTIONS

Handle assay specimens, positive and negative controls as if they were potentially infectious agents.
Wear laboratory coats and disposable gloves while performing the assay. Discard gloves in biohazard waste-bags. Wash hands thoroughly afterwards.
Autoclave all used and contaminated materials at 121 °C, 15 psi for 30 minutes before disposal. Alternatively, decontaminate materials in 5% sodium hypochlorite solution for 30-60 minutes.
Wipe any spills promptly with 1% sodium hypochlorite solution.
The stopping solution is strong acid. Wipe spills immediately. Flush the area of the spill with water. If the stopping solution contacts the skin or eyes, flush with copious amounts of water and seek medical attention.

8 STORAGE AND STABILITY

If kept at 2 to 8 °C, all the test reagents are stable until the expiry date printed on the box.
When the aluminum bag has been opened, the unused strips can be safely stored at 2-8 °C in the sealable plastic pouch along with the silica gel placed inside.
After using a portion of the test reagents: conjugate, TMB, substrate, concentrated washing solution or controls, the remaining contents are stable until the expiry date, if kept at 2-8 °C sealed in the original vials.

9 NECESSARY PREPARATIONS

Working Substrate Solution

The working substrate solution is a 1:1 combination of the substrate with the TMB. For every two strips to be tested, add 1 ml of the TMB to 1 ml of the substrate as shown in the following table:

Note:

1. Do not mix all of the HIV-1/HIV-2 EIA Substrate Buffer with the TMB solution; extra reagents are provided.
2. It is recommended that the working substrate solution should be used in 20 minutes.
3. The working substrate solution should be colorless. A distinct blue color indicates that the solution is contaminated. Discard the working substrate solution and prepare fresh solution in a clean container.

Number of strips to be used	2	4	6	8	10	12
Amount of HIV-1/HIV-2 Substrate Buffer (ml)	1	2	3	4	5	6
Amount of HIV-1/HIV-2 TMB Solution (ml)	1	2	3	4	5	6

Wash Solution

The wash solution (1X) is a 1:25 dilution of the EIA Wash Solution Concentrate (25X) provided with the kit.
Prepare wash solution (1X) as needed by adding one part concentrate (25X) to twenty-four parts deionized water.
The wash solution (1X) can be stored at room temperature for up to 1 week.

Note: *Crystals may form when Wash Solution Concentrate is stored at 2-8°C. This must be dissolved by warming to 37°C prior to use.*

10 PROCEDURE

1. Bring all reagents except the Conjugate to room temperature before beginning the assay procedure.
2. Remove microplate from the aluminium bag, put unused strips and desiccant into the plastic pouch and reseal the pouch.
3. Shake specimen and control vials before use.
4. Add 100 µl of Negative Control to each of the two wells, use a clean pipette tip for addition.
5. Add 100 µl of Positive Control to each of the three wells, use a clean pipette tip for addition.
6. Using pipette, introduce 100 µl of specimen to the assigned wells. In every test leave two wells as blank and do not add specimen or conjugate to these two wells. Use a clean pipette tip for each specimen.
7. Cover the microplate with the plate cover to minimize evaporation and incubate the plate for 60 minutes at 37°C.
8. Bring the Conjugate from 2-8°C storage immediately prior to use.
9. Carefully remove the plate cover and aspirate the fluid from each well into a biohazard container. Prior to disposal, make sure enough disinfectant is added to the container.
10. Wash the microplate five times with diluted wash solution. Aspirate the wash solution each time, after the last washing, blot the inverted plate on absorbent paper towels.

11. Add 100 µl of working conjugate solution to each well containing specimen or control.
12. Cover the plate with a fresh plate sealer and incubate the plate at 37℃ for 30 minutes.
13. Prepare working substrate solution as described before.
14. Remove and discard the plate cover. Repeat the wash procedure as in step 10.
15. Add 100 µl of the working substrate solution per well. Cover the plate with a fresh sealer and incubate the plate for 10 minutes at 37°C.
16. Carefully remove the plate sealer and add 50 µl of stopping solution to each well to terminate the reaction.
17. Read the absorbance for each well at 450 nm. If a dual filter instrument is used, the reference wavelength should be 620 nm.

Note:

1. Once the assay has been started, it should be completed without interruption.
2. Absorbance should be read within 1 hour of the addition of the Stopping Solution.
3. Do not use the microwell washer to aspirate acid and do not aspirate acid into bleach.

11 RESULTS

The presence or absence of antibodies to HIV-1 and/or HIV-2 is determined by relating the absorbance value of the specimen to the cutoff value. The cutoff value is 10% of the mean absorbance value of the Positive Controls.

1. <u>A run is valid if all the following requirements are met:</u>

1) The full complement of Blanks, Positive and Negative Controls must be included in each assay.
2) Blank values must have an absorbance <0.100
3) Negative Control values must have an absorbance <0.080 after subtracting the Blank.
4) Anti-HIV-1 Positive Control value must have absorbance >0.800 after subtracting the Blank.

2. <u>Calculation of Control</u>

Mean of the Positive Controls (PCx)

Determine the mean of Positive Controls as shown in the example below:

Positive Control Sample Time of repeat	Absorbance
1	1.125
2	1.330
3	1.227
Mean of Positive Controls (PCx)	1.227

3. Calculation of the Cut-off Value (COV)

The cut-off value is 10% of the mean of Positive Controls. Calculate the cut-off value as shown in example below:

PCx=1.227

Cut-off Value= 1.227 x 10 % = 0.123

4. Calculation of the Specimen

Calculate the absorbance for each specimen by subtracting the value of Blank from the optical density (OD)-value of each specimen. If the Blank subtracting process is performed by the microplate reader, omit this step. Determine the test result of specimen as shown in the example below:

Specimen Number	Absorbance	OD/COV	
1	0.069	0.069/0.123=0.561	<1.00
2	0.482	0.482/0.123=3.919	>1.00

In the above examples, Specimen No.1 (0.069) is negative and Specimen No.2 (0.482) is positive for antibodies to HIV-1and/or HIV-2 when compared to the cutoff value of 0.123.

12 INTERPRETATION OF THE RESULTS

1. Specimens with absorbance values (OD) less than the cutoff value (COV) (i.e. OD/COV<1.00) are considered to be negative.
2. Specimens with initial absorbance greater than or equal to the cutoff value (i.e. OD/COV>1.00) are considered initially positive by the criteria of this EIA and should be retested in duplicate before interpretation.
3. Specimens found positive on retesting may be interpreted to be repeatedly positive for antibodies to HIV-1 and/or HIV-2 by the criteria of this EIA.
4. Initially reactive specimens which are negative in both wells on the repeat test are considered negative for antibodies to HIV-1 and HIV-2.
5. Specimens which are repeatedly positive in this EIA should be further tested by additional, more specific tests.

13 LIMITATION OF THE PROCEDURE

Repeatedly reactive results by HIV 1+2 Ab ELISA are presumptive evidence of containing antibodies in the specimen. AIDS and AIDS-related conditions are clinical syndromes and their diagnosis can only be established clinically. Testing alone cannot be used to diagnose AIDS, even if the recommended investigation of reactive specimens suggests a high probability that the antibody to HIV-1/HIV-2 is present.

The primary use of the HIV 1+2 Ab ELISA is to screen blood and plasma donations so that units containing antibody can be identified and eliminated, or restricted to further manufacturing into non-injectable products.

A negative test result at any point in the investigation of individual subjects does not preclude the possibility of exposure to or infection with HIV-1/HIV-2.

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SYMBOLS USED WITH DRG ASSAYS

Symbol	English	Deutsch	Français	Español	Italiano
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las instrucciones de uso	Consultare le istruzioni per l'uso
CE	European Conformity	CE-Konfirmitäts-kennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
LOT	Lot. No. / Batch code	Chargen-Nr.	Numéro de lot	Número de lote	Numero di lotto
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Température de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Vertreiber	Distributeur	Distribuidor	Distributore
Content	Content	Inhalt	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità

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