

Instructions for use

For *in vitro* diagnostic use only

HIV EIA

A solid-phase enzyme immunoassay for the detection of antibodies to HIV in human serum or plasma.

Product no. 61 11 011 (Microstrips®, 96 wells)
Product no. 61 11 012 (Microstrips®, 480 wells)
Product no. 61 11 013 (Microstrips®, 960 wells)

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INTENDED USE

Ani Lab systems' 3rd generation HIV EIA is an *in vitro* enzyme immunoassay for the detection of antibodies to HIV in human serum or plasma.

INTRODUCTION

The first case of acquired immunodeficiency syndrome (AIDS) was reported in June 1981 (1). Epidemiological studies have shown that the cause of this deadly disease is a human retrovirus, now known as human immunodeficiency virus type 1 (HIV-1) (2-5). In 1986 a new human retrovirus, named human immunodeficiency virus type 2 (HIV-2) was isolated from patients with AIDS in West Africa (6-7).




Exposure to body fluids such as semen, blood and cervical secretions is necessary for the transmission of HIV. Consequently, HIV 1 / 2 risk groups include sexually active men and women, blood transfusion recipients, haemophilia patients, intravenous drug abusers who share

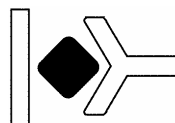
needles and infants of infected mothers (8-9). The majority of the antibodies found in individuals with HIV infection are to the viral envelope proteins gp 120 and gp41 of HIV-1 and gp120 and gp36 of HIV-2 (7, 10-15).


Ani Lab systems' 3rd generation HIV EIA uses a combination of synthetic peptides derived from highly immunoreactive sites of HIV-1 and HIV-2 structural proteins and HIV-1 core protein. Ani Lab systems' 3rd generation HIV EIA was developed to detect antibodies to HIV-1 gp120, gp41 and p24 and HIV-2 gp36 in human serum and plasma. Ani Lab systems' 3rd generation HIV EIA detects subtype-O of HIV-1.

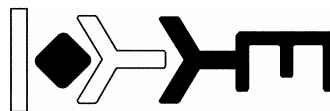
PRINCIPLE OF THE TEST

The principle of Ani Lab systems' HIV EIA kit is based on an indirect solid-phase enzyme immunoassay with horseradish peroxidase as the marker enzyme. The assay proceeds according to the following reactions.

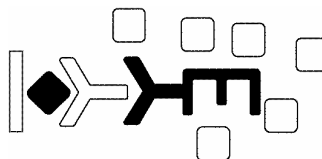
1. When present in patient serum HIV antibodies () combine with HIV peptides () attached to polystyrene surface () of the Microstrip® wells.



2. Residual patient sample is removed by washing and horseradish peroxidase conjugated anti-human IgG (sheep) () is added.



3. Wells are washed and a colourless enzyme substrate (H₂O₂) and chromogen (TMB*) are added. The enzyme reaction of chromogen/substrate produces a coloured end product.



4. Enzyme-chromogen/substrate reaction is terminated with acid (H₂SO₄). The colour intensity is directly related to the concentration of HIV antibodies in a patient sample.

* Tetramethylbenzidine, a non-mutagenic chromogen for horseradish peroxidase (16-17).

KIT CONTENTS

- NOTE:**
- For *in vitro* diagnostic use.
 - The expiration date is printed on each component label and on the package.
 - Reagents are sufficient for 96 wells / 480 wells / 960 wells..
 - Store reagents between +2°C and +8°C.

- 1** MICROSTRIPS®, 12x8 wells / 5 x 12x8 wells / 10 x 12x8 wells)
Coated Microstrips®.
- 2** SAMPLE DILUENT, 25 ml / 4x25 ml / 200 ml
Tris buffered saline with proprietary additives, a blue colouring reagent, and 0.05 % Bronidox® as preservative.
- 3a** NEGATIVE CONTROL, 1.0 ml / 2x1,0 ml / 2.5 ml
HIV antibody negative human serum, a blue colouring reagent, and 0.05 % Bronidox® as preservative.
- 3b** POSITIVE CONTROL 1, 1.0 ml / 2x1,0 ml / 3.5 ml
Heat inactivated HIV-1 antibody positive human serum, a red colouring reagent, and 0.05 % Bronidox® as preservative.
- 3c** POSITIVE CONTROL 2, 1.0 ml / 2x1,0 ml / 3.5 ml
Heat inactivated HIV-2 antibody positive human serum, a red colouring reagent, and 0.05 % Bronidox® as preservative.
- 4** CONJUGATE, 30 ml / 4x30 ml / 250 ml
Buffered salt solution with proprietary additives, a red colouring reagent, horseradish peroxidase conjugated anti-human IgG (sheep) with 0.01 % Thiomersal® as preservative.
- 5** SUBSTRATE BUFFER, 50 ml / 3x50 ml / 250 ml
Citrate-acetate buffer containing 0.005 % hydrogen peroxide and 0.05 % Bromo-Nitro-Dioxane as preservative.
- 6** TMB-CHROMOGEN in DMSO, 1 ml / 3x1 ml / 5.5 ml
3,3',5,5'-Tetramethylbenzidine dissolved in dimethyl-sulfoxide (DMSO). Precautions on page 3. R36/38, S26
- 7** WASHING SOLUTION, 100 ml concentrate / 300 ml concentrate / 500 ml concentrate
Concentrated citrate buffered saline, with proprietary additives, and 0.05 % Bronidox® as preservative.

MICROSTRIP® COVERS, 2 pcs / 10 pcs / 20 pcs

REAGENT PREPARATION

| Reagent | Preparation | Stability of opened/diluted reagents (+2°C to +8°C) |
|--------------------------------------|---|---|
| 1 Coated Microstrips® | Ready for use | 6 months *) |
| 2 Sample diluent | Ready for use | 6 months *) |
| 3a Negative control | Ready for use | 6 months *) |
| 3b Positive control 1 | Ready for use | 6 months *) |
| 3c Positive control 2 | Ready for use | 6 months *) |
| 4 Conjugate | Ready for use | 6 months *) |
| 5 Substrate buffer | Ready for use for the substrate solution | 6 months *) |
| 6 TMB-Chromogen in DMSO | Ready for use for the substrate solution | 6 months *) |
| Substrate solution | Dilute TMB (vial 6) 1 + 49 (1:50) with substrate buffer (vial 5) just prior to use. | Discard unused reagent. A deep blue colour present in the substrate solution indicates that the solution has been contaminated and must be discarded. |
| 7 Washing solution concentrate (10x) | | 6 months *) |
| Washing solution | Dilute the concentrate (vial 7) 1 + 9 (1:10) with distilled water. | 1 month at +4°C or 1 week at room temperature. |

- *) The stability of the opened reagents is the maximum 6 months only if they are stored properly at +2°C to +8°C. However, high environmental temperature and contamination may decrease the stability.

MATERIALS REQUIRED BUT NOT PROVIDED

- Distilled or deionized water, preferably sterile.
- Graduated cylinders, up to 50 ml, for reagent dilutions.
- Vials to store the diluted reagents.
- Precision pipettes (e.g.: one channel 20 - 200 µl, 40 - 200 µl, and multi channel 50-300 µl.)
- Paper towels or absorbent paper.

- Timer, 60 min range.
- Microplate incubator.
- Microplate photometer (plate or Microstrip® reader), 450 nm.
- Microplate washer.
- Sodium hypochlorite solution, free available chlorine 50-500 mg/l (18).
- Disposable gloves.
- Stopping solution (2 M H₂SO₄).

SPECIMEN COLLECTION AND HANDLING

Serum and plasma samples should be refrigerated (+4°C) after collection or, if the test cannot be performed within 48 hours, frozen (-20°C or -70°C, which is preferred).

Samples should not be repeatedly frozen and thawed.

Do not use sodium azide as preservative because it inactivates horseradish peroxidase.

Heat inactivation of serum or plasma (+56°C, 30 min.) may cause an unspecific result.

Microbially contaminated, grossly hemolyzed or hyperlipemic serum and plasma may give erroneous results.

Long storage of serum (frozen over one year) may cause the formation of lipid aggregates. These aggregates may cause an unspecific result.

NOTE: It is not allowed to use diluted or pooled samples!

PRECAUTIONS

For *in vitro* diagnostic use.

WARNING - POTENTIAL BIOHAZARDOUS MATERIAL

Each donor unit of the positive control has been found positive in respect of the presence of HIV markers. Positive controls have been heat inactivated. Because no test or inactivation method can offer complete assurance that human immunodeficiency virus (HIV), hepatitis B virus, or other infectious agents are absent or inactive, these reagents as well as specimens should be handled at the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen in the Centers for Disease Control/National Institutes for Health Manual, "Biosafety in Microbiological and Biomedical Laboratories," 1984.

Discard all materials and specimens as if capable of transmitting infection. The preferred method of disposal is autoclaving for a minimum of one hour at 121°C. Liquid wastes **not containing** acid and neutralized waste may be mixed with sodium hypochlorite in volumes such that the final mixture contains 50-500 mg/l free available chlorine. Allow 30 minutes for decontamination to be completed.

NOTE: Liquid waste containing acid must be neutralized with a proportional amount of base prior to the addition of sodium hypochlorite.

Spills should be wiped up thoroughly using either an iodophor disinfectant or sodium hypochlorite solution. Materials used to wipe up spills should be added to biohazardous waste matter for proper disposal.

Wear disposable gloves while handling specimens and kit reagents. Afterwards wash hands carefully. Never pipette by mouth. Avoid splashing or forming aerosols.

Stopping solution contains 2 M (19.6%) Sulphuric acid. Avoid contact with skin and eyes.

TMB-chromogen (vial 6) is diluted in dimethyl sulfoxide. (DMSO) Avoid contact with skin and eyes.

The melting point of DMSO is +18°C. TMB should be warmed up to +20°C - +25°C and mixed properly before diluting with substrate buffer. Avoid exposure of TMB solution to intense source of light. Oxidizing agents, metallic ions or soap remaining in glassware containers can interfere with the TMB reaction. In order to avoid this problem rinse the glassware thoroughly with 1 N acid (HCl or H₂SO₄) followed by several washes with distilled water before use.

Avoid unnecessary exposure to light. The light sensitive reagents are the chromogen, the conjugate and the substrate buffer, which are packaged in brown glass or nontransparent plastic vials for protection.

All reagents and Microstrips® must be warmed up to +20°C to +25°C before use.

Do not use reagents after the expiration date printed on the label.

Do not mix or interchange reagents from different lots. Do not interchange vial caps.

When removing aliquots from the reagent vials, use aseptic technique to avoid contamination, or erroneous results may occur. Use a new pipette tip for each sample. Do not touch the wells or splash reagents while pipetting. Reusable glassware must be disinfected, washed out and rinsed free of detergents.

Accurate and precise pipetting, as well as following the exact time and temperature requirements, is essential.

Once the assay has been started, all subsequent steps should be performed without interruption. Do not let the wells dry once the assay has been started.

Do not reuse a Microstrip® even if some wells were not used.

Microbial contamination will cause deterioration of diluents and control sera.

Once the Microstrip® foil-package has been opened it has to be resealed tightly and stored at +2°C to +8°C with desiccant.

Once opened the components must be resealed tightly and stored at +2°C to +8°C.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|-------|----------|---|---|---|---|---|---|---|----|----|----|
| A | blank | sample 1 | | | | | | | | | | |
| B | blank | sample 2 | | | | | | | | | | |
| C | 3a | Etc. | | | | | | | | | | |
| D | 3c | | | | | | | | | | | |
| E | 3c | | | | | | | | | | | |
| F | 3b | | | | | | | | | | | |
| G | 3b | | | | | | | | | | | |
| H | 3b | | | | | | | | | | | |

TEST PROCEDURE

Outline of the Procedure

- STEP I** Add 150 µl sample diluent
 Add 50 µl specimens/controls
 Mix carefully while pipetting
 Incubate 30 min., at +37°C
 Wash 5 x 400 µl/well
- STEP II** Add 200 µl conjugate solution
 Incubate 30 min., at +37°C
 Wash 5 x 400 µl/well
- STEP III** Add 200 µl substrate solution
 Incubate 15 min. at RT in dark
- STEP IV** Add 50 µl 2 M H₂SO₄
 Measure at 450 nm

Preliminary Preparations

- NOTE:**
- Wear disposable gloves throughout the procedure!
 - Bring all reagents and Microstrips® to room temperature (+20°C to +25°C) before starting the assay.
 - Prewarm the incubator to +37°C.

Test Procedure

NOTE: Pipette first the samples, and then the controls. Mix the samples and controls carefully with sample diluent while pipetting to avoid gradient.

NOTE: Samples must not be diluted or pooled!

STEP I

1. Pipette 150 µl sample diluent (vial 2) into each Microstrip® well.
2. Add 50 µl sample diluent (vial 2) to wells A1 and B1 for blank.
3. Pipette 50 µl serum or plasma specimens into Microstrip® wells starting from well A2.
NOTE: While pipetting the specimens and controls (items 3-6), insert pipette tip into sample diluent and mix gently to avoid gradient
4. Pipette 50 µl of negative control (vial 3a) into Microstrip® well C1.
5. Pipette 50 µl of positive control 2 (vial 3c) into two Microstrip® wells: D1 and E1.
6. Pipette 50 µl of positive control 1 (vial 3b) into three Microstrip® wells: F1, G1 and H1.
7. Cover the Microstrip® wells with Microstrip® cover. Incubate for 30 minutes at +37°C.

WASHING

Washing may be performed manually or with a washer.

8. Empty the wells into a suitable biohazard container or aspirate the well contents with a washer.
9. Add 400 µl of washing solution (diluted vial 7) into each well. Soak at least 10 seconds.
10. Empty the wells.
11. Repeat the washing cycle five times in total.

STEP II

1. Pipette 200 µl of conjugate (vial 4) into each well. Cover the Microstrip® wells with Microstrip® cover. See NOTE below.
2. Incubate 30 minutes at +37°C.

NOTE: Avoid contamination. Use clean reagent reservoirs, any remaining detergent may cause deterioration. Discard any unused conjugate solution, do not pour it back to the vial.

NOTE: Avoid contamination. Use separate pipette to dispense conjugate and stopping reagent.

WASHING

Wash the wells five times as in items 8 - 11 in STEP I.

STEP III

1. Add 200 µl of the substrate solution (vials 5 and 6) into each well. See NOTE below.

NOTE: The chromogen should be warmed up to +20°C - +25°C and mixed properly before diluting with substrate buffer (see page 3).

Avoid contamination of substrate solution: do not touch the walls of the wells with pipette tips when adding substrate.

2. Incubate 15 minutes at room temperature in a dark place.

STEP IV

1. Stop the enzyme-substrate reaction by adding 50 μ l of 2 M H₂SO₄-solution into each well. See NOTE below.

NOTE: When stopping reagent is added the blue colour of positive reaction should turn yellow. Green colour indicates that the stopping reagent is not properly mixed in the well. If needed mix the wells before measuring.

NOTE: The use of an 8-channel multipipetting device is recommended for improved efficiency and precision.

Reading of the Results

Measure the absorbances immediately at 450 nm.

Blank the photometer at 450 nm against air. Most microplate readers can be blanked against the reagent blank. This means that the absorbance of the reagent blank is automatically subtracted from the absorbance of the samples. However, the absorbance of the reagent blank has to be measured first to check that it falls within the quality control values.

RESULTS

Quality Control Values

Before calculating the results, make sure that the absorbance values obtained for the reagent blank and controls fall within the quality control guidelines.

HIV positive 1 and 2 and negative controls are provided in each kit. The negative control should be included in single, the positive control 2 in duplicate and the positive control 1 in triplicate in each test run. Expected absorbance values for these controls are shown in Table 1. If the blank and the controls do not give expected values, the results are invalid and the specimens should be **retested**.

Two values of positive control 1 must fall within the expected values for the assay to be valid.

Table 1. Quality control values

| QC Sample | Expected value at 450 nm (in absorbance units) |
|-------------------------|--|
| Reagent blank | ≤ 0.10 |
| Negative control (3a) | $\leq 0.15 \times$ |
| Positive control 1 (3b) | $0.70 \leq \text{Apc1} < 2.00 \times$ |
| Positive control 2 (3c) | $> 0.50 \times$ |

\times) The absorbance of the reagent blank has already been subtracted from these values.

Calculation of the Results

Abbreviations:

A = Absorbance
 Arb = Mean absorbance of the reagent blank.
 Apc1 = Mean absorbance of the positive control 1 (3b).
 CO = The cut-off value in absorbance units.

When the microplate reader is blanked against the reagent blank use the following formula for cut-off:

$$\text{CO} = 0.3 \times \text{Apc1}$$

When the microplate reader is NOT blanked against the reagent blank, the cut-off formula is:

$$\text{CO} = 0.3 \times (\text{Apc1} - \text{Arb}) + \text{Arb}$$

Example:

| QC Sample | Mean of the measured absorbances at 450 nm |
|--------------------|--|
| Reagent blank | 0.04 |
| Positive control 1 | 1.37 |

$$\text{CO} = 0.3 \times (1.37 - 0.04) + 0.04 = 0.44$$

Interpretation of the Results

Result Interpretation

$< \text{CO}$ A negative result means that the sample tested either contains no antibodies to HIV or the antibody level is below the detection limit of the test kit. With negative test results, when infection is suspected, it is advised to repeat the test with a new serum sample taken 2-4 weeks later.

$\geq \text{CO}$ An initially reactive test result has to be retested. Only after receiving a repeatedly reactive result, the sample may be presumed to contain antibodies to HIV. The result should be verified with a recognized confirmatory test.

As with other immunoassays, occasional false positive results may occur, which are in most instances non-repeatable. It is therefore recommended to retest all samples giving an initially positive result.

PERFORMANCE CHARACTERISTICS

Reproducibility

Table 1. Within run reproducibility

| Sample | replicates n | mean abs | mean s/c | sd (s/c) | CV % (s/c) |
|----------------------------|-----------------|-------------|-------------|-------------|---------------|
| HIV-1 antibody positive | 24 | 0.74 | 2.27 | 0.20 | 9 |
| HIV-2 antibody positive | 24 | 0.50 | 1.53 | 0.09 | 6 |
| positive control 1 | 11 | 1.01 | 3.09 | 0.22 | 7 |
| positive control 2 | 10 | 0.93 | 2.86 | 0.13 | 4 |

Table 2. Between run reproducibility

| Sample | replicates (within run) | run n | mean abs | mean s/c | sd (s/c) | CV % (s/c) |
|----------------------------|----------------------------|-------|----------|-------------|----------|------------|
| HIV-1 antibody positive | 4 | 8 | 1.26 | 3.43 | 0.11 | 3 |
| HIV-2 antibody positive | 4 | 8 | 1.44 | 3.91 | 0.21 | 5 |
| positive control 1 | 3 | 8 | 1.23 | 3.33 | 0.00 | 0 |
| positive control 2 | 2 | 8 | 1.20 | 3.25 | 0.08 | 2 |

Summary of the specificity studies

HIV antibody negative serum or plasma samples, n=2197, have been tested, table 3.

Table 3. Specificity

| Origin of samples | number of samples tested | reactive | specificity % |
|---------------------------------------|-----------------------------|----------|------------------|
| blood donor, plasma, Finland | 1000 | 5 | 99.5 |
| outpatient clinics, serum, Finland | 1000 | 5 | 99.5 |
| sera, Africa | 197 | 1 | 99.5 |

Summary of the sensitivity studies

HIV-1 antibody positive, n=202, and HIV-2 antibody positive, n=289, serum or plasma samples have been tested, table 4.

Table 4. Sensitivity

| Origin of samples | number of samples tested | reactive | sensitivity % |
|-------------------------------------|-----------------------------|----------|------------------|
| HIV-1 antibody positive, Finland | 200 | 200 | 100 |
| HIV-1 subtype-O positive, BBI | 2 | 2 | 100 |
| HIV-2 antibody positive, Africa | 289 | 289 | 100 |

TROUBLE SHOOTING

| BLANK HAS TOO HIGH ABSORBANCE VALUES | |
|--|------------------------------|
| Cause/Error | Remedy |
| 1. Substrate solution is contaminated | Use clean containers |
| 2. Contamination, spills from other wells | Avoid contamination |
| 3. Washing solution concentrate has not been diluted correctly | Should be diluted 1:10 (1+9) |
| 4. Poor washing | Check your washer |

| POSITIVE CONTROL HAS TOO HIGH ABSORBANCE VALUES | |
|--|--|
| Cause/Error | Remedy |
| 1. Substrate solution is contaminated | Use clean containers |
| 2. Interchange of controls from different lots | Do not mix or interchange reagents from different lots |
| 3. The pipetted volume is too high | Volume should be 50 μ l |

| POSITIVE CONTROL HAS TOO LOW ABSORBANCE VALUES | |
|---|--|
| Cause/Error | Remedy |
| 1. Interchange of controls from different lots | Do not mix or interchange reagents from different lots |
| 2. The pipetted volume is too low | Volume should be 50 μ l |
| 3. The volume of sample diluent is too high | Volume should be 150 μ l |
| 4. The control is not mixed with the sample diluent while pipetting | After dispensing the control rinse the pipette tip with sample diluent |

| NEGATIVE CONTROL HAS TOO HIGH ABSORBANCE VALUES | |
|---|---------------------|
| Cause/Error | Remedy |
| 1. Contamination, spills from other wells, interchange of the vial caps | Avoid contamination |

| ALL ABSORBANCE VALUES ARE VERY HIGH | |
|--|--|
| Cause/Error | Remedy |
| 1. Interchange of reagents from different lots | Do not mix or interchange reagents from different lots |
| 2. Substrate solution is contaminated | Use clean containers |
| 3. Washing solution concentrate has not been diluted correctly | Should be diluted 1:10 (1+9) |
| 4. Poor washing | Check your washer |
| 5. Contaminated solution containers | Use clean containers |
| 6. Deterioration of reagents | Use aseptic technique. Do not pour used reagents back to vials |

| ALL ABSORBANCE VALUES VERY LOW | |
|---|---|
| Cause/Error | Remedy |
| 1. Reagent solutions used after they have expired | Do not use reagents after the expiration date |
| 2. The reagent have not been warmed up to the room temperature | Should be +20°C - +25°C when starting the assay |
| 3. Substrate buffer and chromogen are not mixed in correct ratio | TMB should be diluted 1:50 (1+49) in substrate buffer |
| 4. TMB chromogen have been too cold when diluting with substrate buffer | Should be +20°C - +25°C |
| 5. TMB chromogen have not been mixed properly before diluting | Mix well before diluting, but do not vortex |
| 6. Once opened microtiter plate foil package has not been resealed tightly and stored properly with desiccant | Once opened microtiter plate foil package has to be resealed tightly and stored properly with desiccant |
| 7. Interchange of reagents from different lots | Do not mix or interchange reagents from different lots |
| 8. Substrate solution is exposed to direct sunlight | Avoid unnecessary exposure to light |
| 9. Stopping solution has not been mixed properly before measurement | Mix the plate before measuring |
| 10. Deterioration of reagents | Use aseptic technique. Do not pour used reagents back to vials |
| 11. Contamination of conjugate by human serum or plasma (usually from samples) | Even one microliter of human serum/plasma is enough to inhibit as much as 1 liter of conjugate. Never pour used conjugate back to vial. |

| POOR SPECIFICITY | |
|--|--|
| Cause/Error | Remedy |
| 1. Washing solution concentrate has not been diluted correctly | Should be 1:10 (1+9) |
| 2. Salt crystals in the washing solution concentrate have not been redissolved before diluting | Redissolve the salt crystals before diluting by warming and mixing the concentrate |
| 3. Poor washing | Check your washer |
| 4. Too low positive control value | See: positive control has too low absorbance value |

| POOR SENSITIVITY | |
|---|---|
| Cause/Error | Remedy |
| 1. Too high positive control value | See: positive control has too high absorbance value |
| 2. Sample serum/plasma is not mixed properly with sample buffer | While pipetting mix the sample with sample buffer |
| 3. Frozen samples have not been mixed properly after thawing | Mix well before pipetting |

| | |
|---|--------------------------------|
| 4. Stopping solution has not been mixed properly before measurement | Mix the plate before measuring |
| 5. Samples have been diluted or pooled | Use undiluted samples |

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