HIV-1 IC_x/CR_x Kit

Reagents for Immune Dissociation and Reactivity Confirmation

This product was manufactured in a facility which has a Quality Management System that is certified as being in compliance with ISO 13485.

ZMC Catalog #: 0801096

FOR RESEARCH USE ONLY. Not for *in vitro* Diagnostic Use

INTENDED USE

The RETRO-TEK HIV-1 IC_x/CR_x Kit is supplied for research purposes only. It is not intended for use in the diagnosis or prognosis of disease or for screening and may not be used as a confirmatory test in diagnostic situations.

HIV-1 p24 antigen may be detectable at the onset of an infection with the Human Immunodeficiency Virus Type 1 (HIV-1), prior to seroconversion and late in infection as the disease progresses to AIDS. However, during the ARC (AIDS Related Complex) phase of the disease, p24 antigen assays have had limited utility, since the majority of patients produce both anti-p24 antibody and p24 antigen and form immune complexes. These immune complexes may either interfere with or prevent the measurement of p24 antigen by conventional immunoassays[2,3].

The RETRO-TEK HIV-1 IC_x/CR_x Kit complements the RETRO-TEK HIV-1 p24 Antigen ELISA (ZMC Catalog #: 0801111). The IC_x/CR_x Kit contains two sets of reagents, each of which may provide supplemental information that enhances the detection of p24 antigen in serum or plasma:

- The Immune Complex Dissociation (IC_x) Reagents may identify serum or plasma specimens in which p24 antigen is masked by antibody.

- The Confirmation Reagents (CR_x) may confirm the reactivity of positive specimens.

REAGENTS

Materials Supplied:

- **IC_x Acid, 25 ml**: Contains glycine-HCl. (Reagent A)
- **IC_x Base, 25 ml**: Contains tris-HCl and sodium azide. (Reagent B)
- **IC_x Positive Control, 1 ml**: Contains human source material with p24 immune complexes (heat inactivated) and sodium azide. (Reagent C)
- **IC_x/CR_x Negative Control, 1 ml**: Contains human source material non-reactive for antibodies to HIV-1 and non-reactive for HIV-1 p24 antigen and sodium azide. (Reagent D)
- **CR_x Control Reagent, 1ml**: Contains human source material non-reactive for antibodies to HIV-1 and non-reactive for HIV-1 p24 antigen, Triton X-100® and sodium azide. (Reagent E)
- **CR_x Neutralization Reagent, 1 ml**: Contains human source material with antibodies to HIV-1 (heat inactivated), Triton X-100® and sodium azide. Non-reactive for HIV-1 p24 antigen.

® Triton X-100 is a registered trademark of Union Carbide Chemicals and Plastics Co., Inc.

Storage:

Store all kit reagents at 2° - 8°C. DO NOT FREEZE. When stored properly the kit is stable until the date indicated on the box label.

Materials Required but Not Supplied:

- **Lysing Buffer, HIV-1 p24 Antigen Standard** and **Assay Diluent** from RETRO-TEK HIV-1 p24 Antigen ELISA
- **RETRO-TEK HIV-1 p24 Antigen ELISA**
- **Disposable gloves**
- Validated adjustable **micropipettes**, single and multichannel
- **Test tubes and racks** for preparing specimen and control dilutions
- Graduated **cylinders** and assorted **beakers**
- Validated automatic **microplate washer** or manual vacuum aspiration equipment
- Validated **incubator** for 37°C ±1°C
- Validated **microplate reader**
- **Timer**
- **1% sodium hypochlorite** as disinfectant. May be prepared from household bleach
- **Distilled or deionized water**

PRECAUTIONS

FOR RESEARCH USE ONLY. Not For *in vitro* Diagnostic Use.

- Prior to performing the assay, carefully read all instructions.
- Use universal precautions when handling kit components and test specimens.*
- To avoid cross-contamination, use separate pipet tips for each specimen.
- Do not interchange components of this kit with any other kits or reagents.
- Disposal: When testing potentially infectious human specimens, adhere to all applicable local, state and federal regulations regarding the disposal of biohazardous material.
- Human source material used in the manufacture of the HIV-1 p24 Detector Antibody, IC_x Positive Control, IC_x /CR_x Negative Control, CR_x Control Reagent and CR_x Neutralization Reagent has been tested and found negative for Hepatitis B surface antigen. The viral lysate used to prepare the HIV-1 p24 Antigen Standard has been inactivated by chemical disruption and heating. Handle these reagents as if capable of transmitting infectious agents.

* from MMWR, June 24, 1988, Vol. 37, No. 24, pp. 377-382, 387-388.

1. IMMUNE COMPLEX DISSOCIATION

TEST PROCEDURE

Allow all reagents to reach room temperature before use. Label test tubes to be used for preparation of Positive Control, Negative Control and specimens.

Step 1: Pipet 75 µl of IC_x Positive Control and 75 µl of IC_x Acid into the appropriate tubes and mix. Prepare IC_x/CR_x Negative Control and all serum or plasma specimens in the same manner.

Step 2: Incubate for 1 hour at 37°C (±1°C).

Step 3: During incubation, prepare all reagents necessary to perform the RETRO-TEK HIV-1 p24 Antigen ELISA.

Step 4: Pipet 75 µl of IC_x Base reagent into each sample or control and mix.

Step 5: Pipet 25 µl of Lysing Buffer into each test tube and mix.

Step 6: Immediately assay each specimen using the RETRO-TEK HIV-1 p24 Antigen ELISA as described on **Page 4** of the p24 ELISA Product Insert. Omit Step 1 since Lysing Buffer has already been added.

IMMUNE COMPLEX DISSOCIATION PROCEDURAL FLOW CHART

IC_x ACID REAGENT, CONTROLS AND SPECIMENS

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INCUBATE 1 HOUR AT 37°C ± 1°C

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PIPET IC_x BASE REAGENT

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PIPET LYSING BUFFER

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PROCEED WITH RETRO-TEK HIV-1 p24 ANTIGEN ELISA

2. CONFIRMATION OF p24 ANTIGEN POSITIVE SPECIMENS BY NEUTRALIZATION

PRINCIPLE OF TEST

Samples found reactive in the RETRO-TEK HIV-1 p24 Antigen ELISA should be confirmed by neutralization. Each reactive sample is incubated separately with the CR_x Neutralization and the CRx Control reagents prior to analysis in the RETRO-TEK HIV-1 p24 Antigen ELISA. During the incubation, p24 antigen present in the sample will complex with the anti-p24 antibody contained in the CRx Neutralization Reagent; conversely, no antigen-antibody complexing will occur in the sample incubated with the CR_x Control Reagent. When tested in the ELISA, non-complexed antigen in the sample will be captured by the anti-p24 coated microplate; complexed antigen will not be captured by the anti-p24 coated microplate, resulting in reduction of the optical density.

In order to perform confirmation testing, samples must have a concentration of p24 antigen less than 125 pg/ml. Specimens containing more than 125 pg/ml must be diluted to a concentration of approximately 50 to 125 pg/ml prior to testing.

For confirmation of p24 antigen for specimens Initially reactive without IC_x treatment, proceed with Steps 1 to 4 on this page. For specimens initially reactive after IC_x treatment, proceed with Steps 1 to 6 on **Page 6.**

TEST PROCEDURE WITHOUT IC_x TREATMENT

Allow all reagents to reach room temperature before use. For the following procedure, consult Table 1 for the appropriate test tube labels, reagents and volumes.

Step 1: Prepare the Antigen Positive Control (AgPC) by pipetting 25 µl of the HIV-1 p24 Antigen Standard into 975 µl of Assay Diluent (62.5 pg/ml final concentration) from the RETRO-TEK HIV-1 p24 Antigen ELISA.

Step 2: Pipet 225 µl of Antigen Positive Control (AgPC), 225 µl of IC_x/CR_x Negative Control (Reagent D), or 225 µl of each specimen into their respective test tubes. Pipet 25 µl of Lysing Buffer from the RETRO-TEK HIV-1 p24 Antigen ELISA into each test tube. Pipet 30 µl of the CR_x Control Reagent (Reagent E) or 30 µl of the CR_x Neutralization Reagent (Reagent F) into their respective test tubes and mix.

Step 3: Incubate for 1 hour at 37°C (±1°C).

Step 4: Immediately assay each specimen using the RETRO-TEK HIV-1 p24 Antigen ELISA as described on **Page 4** of the p24 ELISA Product Insert. Omit Step 1 since Lysing Buffer has already been added.

Information regarding test validity requirements, calculations and interpretations of results is contained in the p24 ELISA Product Insert.

Table 1

Preparation of Specimens and Controls (without Immune Complex Dissociation)

Tube Label	Reagents (µl) AgPC	D	Specimen	LB	E	F	
PC_C	225	0	0	25	30	0	I
PC_N	225	0	0	25	0	30	N
NC_C	0	225	0	25	30	0	C
NC_N	0	225	0	25	0	30	U
$S1_C$	0	0	225	25	30	0	B
$S1_N$	0	0	225	25	0	30	A
$S2_C$	0	0	225	25	30	0	T
$S2_N$	0	0	225	25	0	30	E
etc.							

Legend

Reagent	AgPC	Antigen Positive Control prepared in Step 1
Reagent	D	IC_x/CR_x Negative Control
Reagent	LB	Lysing Buffer
Reagent	E	CR_x Control Reagent
Reagent	F	CR_x Neutralization Reagent

NEUTRALIZATION PROCEDURAL FLOW CHART

Without ICx Treatment

PREPARE SPECIMENS, CONTROL AND REAGENTS

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PIPET SPECIMENS, CONTROLS AND REAGENTS

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INCUBATE 1 HOUR AT 37°C ± 1°C

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PROCEED WITH RETRO-TEK HIV-1 p24 ANTIGEN ELISA

TEST PROCEDURE WITH ICx TREATMENT

Allow all reagents to reach room temperature before use. For the following procedure, consult Table 2 for the appropriate test tube labels, reagents and volumes.

Step 1: Pipet **75 µl** of **IC_x Positive Control** (Reagent C), **75 µl** of **IC_x/CR_x Negative Control** (Reagent D) or 75 µl of each specimen into their respective test tubes. Pipet **75 µl** of **IC_x Acid** (Reagent A) into their respective test tubes and mix.

Step 2: Incubate for 1 hr. at 37°C (±1°C).

Step 3: Pipet **75 µl** of **IC_x Base** (Reagent B) into each test tube and mix.

Step 4: Pipet **25 µl** of **Lysing Buffer** (Reagent LB) from the RETRO-TEK HIV-1 p24 Antigen ELISA into each test tube and mix.

Step 5: Pipet **10 µl** of the **CR_x Control Reagent** (Reagent E) or **10 µl** of the **CR_x Neutralization Reagent** (Reagent F) into their respective tubes and mix.

Step 6: Incubate for 1 hr. at 37ºC (±1°).

Step 7: Immediately assay each specimen using the RETRO-TEK HIV-1 p24 Antigen ELISA as described on **Page 4** of the p24 ELISA Product Insert. Omit Step 1 since Lysing Buffer has already been added.

Information regarding test validity requirements, calculations and interpretations of results is contained in the p24 ELISA Product Insert.

Table 2

Preparation of Specimens and Controls (with Immune Complex Dissociation)

Tube Label	Reagent (µl) C	D	Specimen	A		B	LB	E	F	
PC_C	75	0	0	75	I	75	25	10	0	I
PC_N	75	0	0	75	N	75	25	0	10	N
NC_C	0	75	0	75	C	75	25	10	0	C
NC_N	0	75	0	75	U	75	25	0	10	U
$S1_C$	0	0	75	75	B	75	25	10	0	B
$S1_N$	0	0	75	75	A	75	25	0	10	A
$S2_C$	0	0	75	75	T	75	25	10	0	T
$S2_N$	0	0	75	75	E	75	25	0	10	E
etc.										

Legend

Reagent	C	IC_x Positive Control
Reagent	D	IC_x/CR_x Negative Control
Reagent	LB	Lysing Buffer
Reagent	A	IC_x Acid
Reagent	B	IC_x Base
Reagent	E	CR_x Control Reagent
Reagent	F	CR_x Neutralization Reagent

NEUTRALIZATION PROCEDURAL FLOW CHART

with IC_x Treatment

PREPARE SPECIMENS, CONTROL, AND REAGENTS

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PIPET SPECIMENS, CONTROLS, AND REAGENT A

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INCUBATE 1 HOUR AT 37°C ± 1°C

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PROCEED WITH RETRO-TEK HIV-1 P24 ANTIGEN ELISA

REFERENCES:

1. Morrow, WJW et al. 1986. Circulating immune complexes in patients with acquired immune deficiency syndrome contain the AIDS-associated retrovirus. Clin Immunol Immunopathol. 40: 515-524.
2. Nishanian, P et al. 1990. A simple method for improved assay demonstrates that HIV p24 antigen is present as immune complexes in most sera from HIV-infected individuals. J Inf Dis. 162: 21-28.
3. Bollinger, RC, Jr.et al. 1992. Acid dissociation increases the sensitivity of p24 antigen detection for the evaluation of antiviral therapy and disease progression in asymptomatic human immunodeficiency virus-infected persons. J Inf Dis. 165: 913-916.

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REV.: 05/2007
1004185