DATA SHEET

HIV-1 p24
Extended Range Kit

ZMC Catalog #: 0801137

Gentaur Molecular Products
Voortstraat 49
1910 Kampenhout, Belgium

INTENDED USE

The RETRO-TEK HIV-1 p24 Extended Range 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. It is to be used as an accessory to the RETRO-TEK HIV-1 p24 ELISA.

PRINCIPLE OF THE TEST

Many specimens and tissue culture samples contain HIV-1 viral loads greater than the range of the standard curve of commercially available antigen ELISA's. In order to quantify such samples, a series of expensive and time consuming dilutions must be made to adjust the sample concentration. The RETRO-TEK HIV-1 p24 Extended Range Kit includes reagents that extend the standard curve of the RETRO-TEK HIV-1 p24 ELISA (ZMC catalog #: 0801111) up to 4 ng/ml. Samples containing p24 antigen up to 4 ng/ml may be accurately quantified without extensive serial dilution by kinetic reading of the assay plate.

REAGENTS

Materials Supplied

- Concentrated HIV-1 p24 Antigen Standard (0.3ml): Contains detergent disrupted, heat inactivated viral antigen at a concentration of 80 ng/ml, added protein, detergent and 2-chloroacetamide.
- Substrate (5 tablets): Contains OPD (orthophenylenediamine-HCl)

Materials Required but Not Supplied

- RETRO-TEK HIV-1 p24 ELISA (ZMC catalog #: 0801111)
- Test tubes and racks for preparing specimen and standard dilutions
- Adjustable micropipettes, single and multichannel

- Incubator capable of maintaining 37º± 1º C
- Timer
- Graduated cylinders and assorted beakers
- Automatic microplate washer or manual vacuum aspiration equipment
- 1% sodium hypochlorite as disinfectant. May be prepared from household bleach.
- Distilled or deionized water

Storage:

Store all kit reagents at 2°-8°C.

PRECAUTIONS

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

- OPD is a possible carcinogen. Avoid contact. Use gloves when handling tablets.
- Use Universal Precautions when handling test specimens and when performing this test.*
- Disposal: When examining human source material or other potentially infectious specimens, adhere to all applicable local, state and federal regulations regarding disposal of hazardous materials.
- To avoid cross-contamination, use separate pipet tips for each specimen

*from MMWR, June 24,1988, Vol. 37, No.24, pp. 377-382, 387-8

PREPARATION OF REAGENTS

Extended Range Standard Curve: Prepare a series of seven standards from the Concentrated HIV-1 p24 Antigen Standard.The dilution scheme in Table 1 is recommended.

Table 1
Preparation of HIV-1 p24 Antigen Standard

Standard Number	Concentration of HIV-1 p24 (pg/ml)	Volume of conc. AntigenStandard (µl)	Volume of Assay Diluent (µl)
1	4000	50 µl	950 µl
2	2000	500 µl of #1	500 µl
3	1000	500 µl of #2	500 µl
4	500	500 µl of #3	500 µl
5	250	500 µl of #4	500 µl
6	125	500 µl of # 5	500 µl
7	0	0 µl	500 µl

Any diluted standards remaining after the completion of the assay should be discarded. Do not save diluted reagent.

OPD Substrate Solution: Dissolve 1 OPD tablet in 11.0 ml of the Substrate Buffer supplied in the RETRO-TEK HIV-1 p24 ELISA. Do not make up more than 20 minutes prior to use. Protect from light until use. Use the solution within 15 minutes of its preparation. Do not save diluted reagent.

TEST PROCEDURE

Allow all reagents to reach room temperature before use. Proceed with the RETRO-TEK HIV-1 p24 ELISA according to the product insert making the following changes:

1. Replace the standard curve supplied with the kit with the Extended Range Standard Curve.
2. Replace the liquid Substrate supplied with the p24 kit with the OPD Substrate Tablets.
3. Immediately after addition of OPD Substrate Solution to the plate, read in a kinetic plate reader. DO NOT add stop solution to the plate.

The suggested instrument set-up for kinetic readings is:

Total run time:	15 minutes
Time interval to read:	10 sec
Wavelength:	405 nm
OD limit:	1.0 OD
Lag time:	0

CALCULATION AND INTERPRETATION OF RESULTS

The HIV-1 p24 concentration of unknown samples may be determined by using a point-to-point algorithm, or linear regression. Only readings which fall within the range of the standard curve may be quantified. Use the antigen concentrations as the X values and the mean slope/min as the Y values.

If kinetic reading capabilities are not available, the plate should be read every 2 minutes for 15 minutes. Determine the linearity of the standard curve for every 2 minute time point. Upon examining these curves, select the curve which is most linear in the range of the samples to be quantified. Perform calculations by point-to-point algorithm or linear regression. Use the Antigen concentration as the X values and the OD as the Y values.

TYPICAL STANDARD CURVE: This is an example of a typical standard curve. Variation may occur in individual labs due to pipetting, laboratory and incubator temperatures, etc.

HIV-1 Antigen Standard Concentration	Mean Slope
4.00 ng/ml	57.46
2.00 ng/ml	48.79
1.00 ng/ml	35.23
0.50 ng/ml	24.95
0.25 ng/ml	16.44
0.125 ng/ml	8.96
0.00 ng/ml	0.085

LIMITATIONS OF THE PROCEDURE

Most HIV-1 infected individuals produce antibodies to p24 antigen. The concentration and microspecificity of these antibodies will vary from individual to individual and from bleed to bleed. The observed level of p24 antigen in any specimen containing p24 antibodies may be affected by the following:

- Host antibodies may mask the epitope reactive with capture MAb. Consequently, the optical density may be reduced.

- Host antibodies may mask epitopes that bind to the detector antibody, again reducing the optical density.

After immune complex dissociation and neutralization, host antibodies may recombine with p24 antigen. The recombination of host antibody with antigen is competitive with the capture of p24 by the MAb on the solid phase and thus may remask some of the p24. The rate of recombination is determined by the concentrations of p24 antigen and anti-p24 antibodies, the affinity of the antibodies, as well as temperature and time and is therefore difficult to control. Correlation of results for specimens between repeat test runs and among kits from different manufacturers may be adversely affected by such competitive recombination.

PROCEDURAL FLOW CHART

PREPARE REAGENTS

↓

WASH PLATE

↓

PIPET SPECIMENS, STANDARDS AND CONTROLS

↓

INCUBATE 2 HOURS AT 37º ± 1ºC

↓

WASH PLATE

↓

PIPET DETECTOR ANTIBODY

↓

INCUBATE 1 HOUR AT AT 37º ± 1ºC

↓

WASH PLATE

↓

PIPET STREPTAVIDIN PEROXIDASE SOLUTION

↓

INCUBATE 30 MINUTES AT AT 37º ± 1ºC

↓

WASH PLATE

↓

PIPET SUBSTRATE SOLUTION

↓

READ PLATE AT 405 NM

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