



TDM-Elisa APV / FOS (Amprenavir / Fos Amprenavir)

For *in-vitro* diagnostics use only.

INTRODUCTION

Amprenavir and Fos amprenavir (APV/Fos APV) belong to the class of peptide-mimetic HIV-1 protease inhibitors (PI). Such drugs are employed in HIV infection control treatments (HAART = Highly Active Anti-Retroviral Therapy). The pro-drug Fos amprenavir taken by mouth is quickly and almost completely hydrolysed at the intestinal mucosa level before reaching systemic circulation.

Plasmatic concentrations of protease inhibitors are connected to therapeutic efficacy, but also to toxic side effects.

TDM-Elisa APV/Fos APV is an enzyme-immunoassay for the determination of plasmatic concentration of amprenavir (APV) within the therapeutic range (Re.: Guide-lines). The results of therapeutic drug monitoring (TDM) are for the clinician a very important tool because allow to evaluate altered drug levels as cause of toxicity or uneffective treatment.

SAMPLES

Human plasma

Samples must be stored at 2-8°C and used within 24 hours or aliquoted and frozen (-20°, - 80°C). Avoid repeated freezing and thawing cycles.

FORMAT

96 wells microplate (8 wells strips)

DOSAGE RANGE

0.4 to 5 µg/ml

STORAGE

The kit must be stored at 2-8°C.

SHELF-LIFE

9 months from production

TIME NEEDED FOR THE TEST

1h and 30 min (excluding sample pre-treatment).

NUMBER OF SAMPLES

40 samples in duplicate

CONTENT OF THE KIT

| COMPONENTS | QUANTITY |
|---------------------------------------|--------------|
| Microplate (96 wells) | 12 x 8 wells |
| Antisiero APV | 1x 12ml |
| Enzima APV | 1x 10ml |
| Lopinavir calibrators/ Standard Curve | 7 x 150 µl |
| TMB 10X | 1x 3ml |
| Diluyente del campione APV | 1 x 50ml |
| Development Solution | 1x 30ml |
| Washing Solution 10X | 1x 100ml |
| Stop Solution | 1x 7ml |

INSTRUMENTS NEEDED

Microplate reader with filters at 450 e 620 nm

Microplate washer.

Pipettes (P20 and P1000) and Multichannel Pipette multicanale with 8 tips (volumes from 50 to 300µl)

Microcentrifuge for Eppendorf 1.5 ml tubes.

TEST PROTOCOL

APV/Fos APV is a competitive quantitative enzyme-immunoassay .

APV/ Fos APV is based on the competition between the drug in the patient's plasma and the same drug conjugated with a revealing enzyme; they compete for binding to the same drug-specific polyclonal antibody. A specie-specific solid phase captures the specific antibody. Excess sample and reagents are removed by washing. Detection of the conjugate bound to the solid phase is achieved by adding a chromogenic solution. Enzymatic activity produces a coloured solution whose absorbance can be read on a microplate reader. Absorbance values are inversely proportional to the drug concentration in the sample.

SAMPLE PREPARATION

Mix samples well using a Vortex mixer for 10-15sec.

Dilute plasma samples 1:100 with Sample Diluent.

Vortex mix for 10 –15sec.

For patients with concentrations higher 5 µg (up to 10 µg), it is possibile to dilute 1:200; in this case to obtain the final concentration value the results should be multiplied by the dilution factor 2.

PROCEDURE SUMMARY

Transfer 20 µl of Calibrators and pre-treated samples in the appropriate wells.

Pipette 80 µl of Enzyme- APV and then 100 µl of APV Antiserum in all wells, excluding the blanks.

Incubate for 60 min at RT.

Wash the plate 5 times filling all the wells (about 350µl) with diluted washing solution.

Pipette 200µl of pre-diluted chromogenic solution cromogena in each well with a multichannel pipette.

Incubate 30 min at RT in the dark.

Add 50 µl of Stop Solution in each well.

Read absorbance values at 450 nm on a microplate reader

CALCULATION OF RESULTS

If software is available, use a 4-Parameters Logit-Log.

For manual evaluation, calculate the average of calibrators and samples absorbances and subtract the average blanks value.

Calculate for each well B/B_0 according to the formula:

$$\frac{\text{Average absorbance value of calibr. or Sample} \times 100}{\text{Average absorbance of 0 calibr.}}$$

Read the values on the standard curve.

MANUFACTURER

BioStrands S.r.l.

Via del Follatoio 12

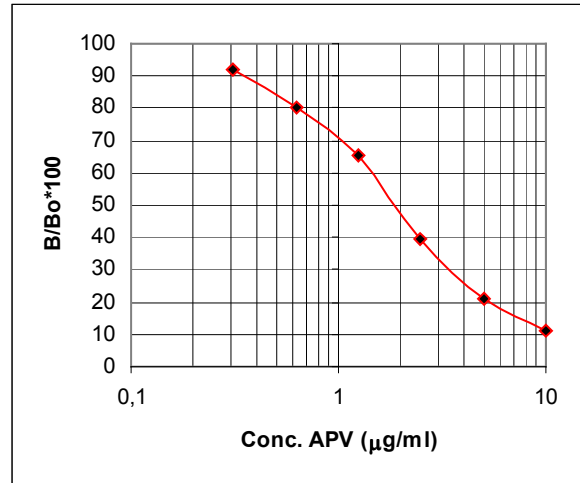
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EXAMPLE OF STANDARD CURVE



BIBLIOGRAPHY

- Guidelines for the use of antiretroviral agents in HIV-1 infected adults and adolescents, Oct 2006
- An enzyme immunoassay (TDM ELISA) compared to high performance liquid chromatography (HPLC) to determine amprenavir plasma concentrations in HIV-infected patients. Seminari E., Donadel E., Gentilini G., Soldarini A., Bastiani E., Galli L., Rinaldi S., Dorigatti F., Castagna A., Lazzarin A., IV Forum SIVIM Gennaio 2006 "Infezione da HIV: Diagnostica avanzata e terapie antiretrovirali innovative" - Milano
- To determine Fos-Amprenavi plasma concentrations in HIV-infected patients: Elisa kit compared to high performance liquid chromatography (HPLC) Gentilini g., Seminari E., Soldatini A., Donadel E., Bastiani E., Rinaldi S., Castagna A., Lazzarin A., Dorigatti F., 7th International congress on Drug Therapy in HIV Infection - Glasgow Nov 2004
- Evaluation of an Elisa Method for measuring Amprenavir in plasma. Montagna M., Bastiani E., Cusato M., Benedetti F., Rinaldi S., Donadel E., Berti F., Meroni V., Ragazzi M., Varsavia Settembre 2003.

| REF | DESCRIPTION | FORMAT |
|------|--------------------------|----------|
| 1678 | TDM-Elisa APV/Fos APV | 96 wells |

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