



## TDM-Elisa LPV ( Lopinavir)

### For *in-vitro* diagnostics use only

#### INTRODUCTION

Lopinavir (LPV) belongs to the class of peptide-mimetic HIV-1 protease inhibitors (PI). Such drug is employed in association with ritonavir (RTV) in HIV infection control treatments: Highly Active Anti-Retroviral Therapy (HAART).

Plasmatic concentrations of protease inhibitors are correlated with therapeutic efficacy, but also to toxic side effects. Concentration levels of antiviral drugs are important to determine if individuals are receiving suitable drug exposures.

TDM-Elisa LPV is an enzyme-immunoassay for the determination of plasmatic concentration of lopinavir (LPV) within the therapeutic range (Re.: Guide-lines). The results of therapeutic drug monitoring (TDM) are a very important tool for the clinician and allow to evaluate altered drug levels as cause of toxicity and side effects or therapeutic failure.

#### 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

1 to 8 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
LPV Antiserum	1x 12ml
LPV Enzyme	1x 10ml
Lopinavir calibrators/ Standard Curve	7 x 150 µl
TMB 10X	1x 3ml
Development Solution	1x 30ml
Washing Solution 10X	1x 100ml
Stop Solution	1x 7ml

#### MATERIALS NEEDED BUT NOT SUPPLIED

Methanol

#### 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

TDM-Elisa LPV is a competitive quantitative enzyme-immunoassay .

TDM-Elisa LPV 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.

Take 100 µl of plasma and dilute with 300 µl of Methanol. Vortex mix for 10 –15sec. and centrifuge 10min at 10,000 x g.

Take 100 µl of clear surnatant and dilute it with 150 µl distilled water. Vortex for 10-15sec. Take 100 µl of this dilution and add 150 µl of a 30% methanol solution. Vortex mix 10-15sec

### PROCEDURE SUMMARY

Transfer 20 µl of Calibrators and pre-treated samples in the appropriate wells.  
 Pipette 80 µl of Enzyme-LPV and then 100 µl of LPV-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 chomogenic solution 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:

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

Read the values on the standard curve.

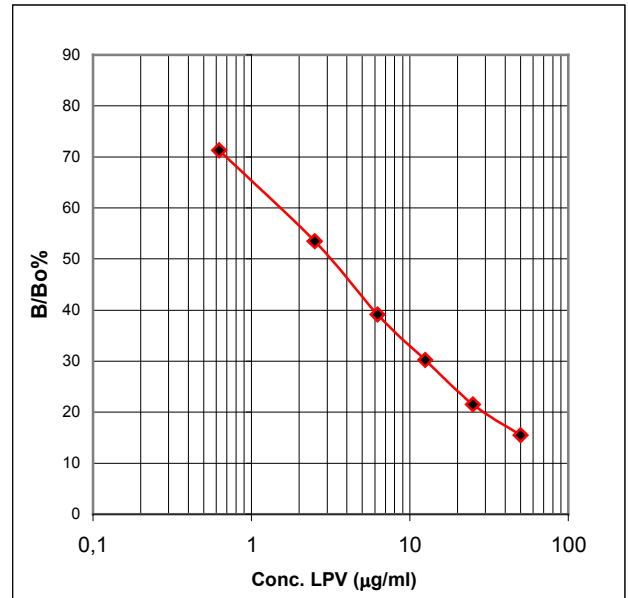
REF	DESCRIPTION	FORMAT
2678	TDM-Elisa LPV	96 wells

### MANUFACTURER

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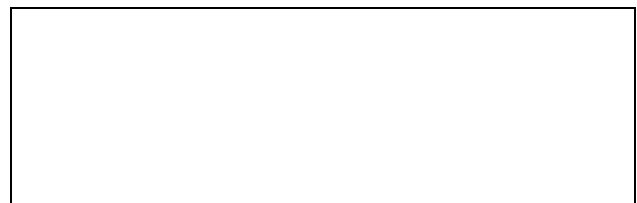


### EXAMPLE OF STANDARD CURVE



### BIBLIOGRAPHY

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- A new ELISA method for the determination of Lopinavir e Ritonavir in human plasma  
 Zanone Poma B, Menzaghi B, Brogгинi V, Mologni D, Bastiani E, Rinaldi S, Galli M<sup>1</sup>, Riva A, Abstract XVI International AIDS conference – Toronto Canada, Agosto 2006
- An enzyme immunoassay for the quantification of plasma and intracellular lopinavir in HIV-infected patients. Azoulay S, Nevers MC, Creminon C, Heripret L, Garraffo R, Durant J, Dellamonica P, Grassi J, Guedj R, Duval D  
 J Immunol Methods. 2004 Dic.; 295(1-2):37-48



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