



## TDM - Elisa RTV booster (Ritonavir booster)

For *in-vitro* diagnostics use only

### INTRODUCTION

Ritonavir (RTV) belongs to the class of peptide-mimetic HIV-1 protease inhibitors (PIs) used in HIV infection control treatments (HAART = Highly Active Anti-Retroviral Therapy).

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

RTV is given at low dosage (booster) complementary to other protease inhibitors because it has been observed that interaction between RTV and other PIs improves the pharmacokinetics of the latter once.

This combination allows reduction and frequency of administration of the second PI, so increasing tolerance and compliance to treatment.

TDM-Elisa RTV booster is an enzyme immunoassay for the determination of plasmatic concentrations of RTV within the therapeutic range when used at low dosage in association with other PIs. The results of the therapeutic drug monitoring (TDM), offer the clinician very important data because allow to monitor drug levels as possible cause of toxicity 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

0.09 a 0.9 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
RTV Antiserum	1x 12ml
RTV Enzyme	1x 10ml
Ritonavir calibrators/ Standard Curve	7 x 300 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 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 RTV-booster 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 chromogen 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 MetOH 30%.  
Mix on a vortex for 10-15sec

### PROCEDURE SUMMARY

Transfer 20 µl of Calibrators and pre-treated samples in the appropriate wells.  
Pipette 80 µl of Conjugate-RTV and then 100 µl of RTV 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 chromogen 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

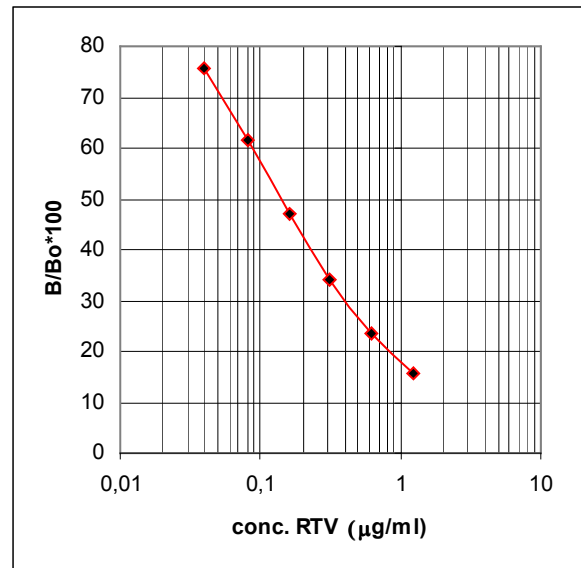
### CALCULATION OF RESULTS

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:

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

Read the values on the standard curve.

### EXAMPLE OF STANDARD CURVE



### BIBLIOGRAPHY

- Guidelines for the use of antiretroviral agents in HIV-1 infected adults and adolescents, Oct 2006
- A new ELISA method for the determination of Lopinavir e Ritonavir booster 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

REF	DESCRIPTION	FORMAT
7678	TDM - Elisa RTV booster	96 wells

### MANUFACTURER

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