



TDM-Elisa NFV (Nelfinavir)

For *in-vitro* diagnostics use only

INTRODUCTION

Nelfinavir (NFV) belongs to the class of peptide-mimetic HIV-1 protease inhibitors (PI). Such drug is employed 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.

TDM-Elisa NFV is an enzyme-immunoassay for the determination of plasmatic concentration of Nelfinavir (NFV) and its major metabolite pharmacologically active M8 (hydroxy-t-butylamide). The results of therapeutic drug monitoring (TDM) are for the clinician a very important tool and 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

0.8 - 3 µg/ml

STORAGE

The kit must be stored at 2-8°C.

SHELF-LIFE

6 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
NFV Antiserum	1x 12ml
NFV Conjugate	1x 10ml
Lopinavir calibrators/ Standard Curve	7 x 300 µl
TMB 10X	1x 3ml
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

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

TDM-Elisa NFV 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 100 µl plasma samples with 300 µl of

Methanol. Vortex mix for 10 -15sec.

Centrifuge 10 min at 10.000x g.

Take 100 µl of clear supernatant and dilute with 150 µl of distilled water. Mix well on a Vortex for 10-15sec.

Take 10 µl of the last dilution and 990µl of 30% Methanol .

PROCEDURE SUMMARY

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

Pipette 80 µl of NFV Conjugate and then 100 µl of NFV Antiserum in all wells, except for the blank wells.

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 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.

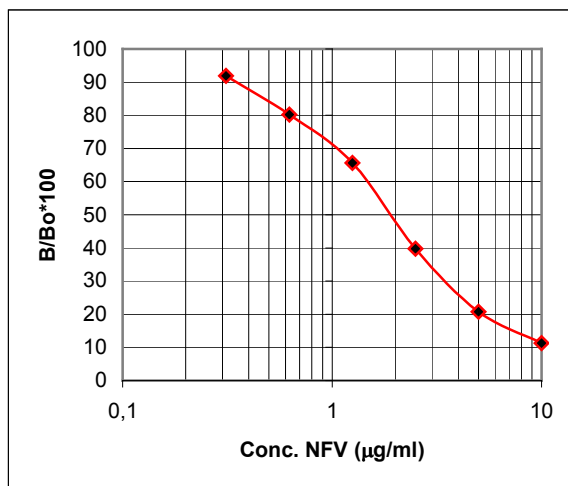
For manual evaluation, calculate the average of calibrators and samples absorbances and subtract the average blanks value.

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

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

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
- Valutazione di un test ELISA per il monitoraggio terapeutico del nelfinavir in pazienti HIV positivi
F. Genco, V. Meroni, A. Uglietti, E. Donadel, S. Rinaldi, E. Bastiani, R. Maserati Abstract AMCLI 2006

REF	DESCRIPTION	FORMAT
3678	TDM-Elisa NFV (Nelfinavir)	96 wells

MANUFACTURER

BioStrands S.r.l.

Via del Follatoio 12

34148 Trieste (Italia)

Tel. +39.040.8992.451

Fax. +39.040.8992.452



Rev. 0;110507

