

TDM-Elisa NFV (Nelfinavir)

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1 INTENDED USE

TDM-Elisa NFV (Nelfinavir) is an enzyme immunoassay for the quantitative colorimetric determination of Nelfinavir and its hydroxy-t-butylamide metabolite (M8) in human plasma samples, as an aid in managing HIV-1 patients' therapy and monitoring compliance.

For research use only.

2 PRINCIPLE OF THE TEST

TDM-Elisa NFV (Nelfinavir) is based on the competition between the drug or its major metabolite (M8) in the plasma sample and the drug conjugated with the detection enzyme for the same binding sites of an anti-Nelfinavir polyclonal antiserum.

The specific antibodies bind to the solid phase to which species-specific antibodies are absorbed.

To the microtiter wells are sequentially added calibrators, samples and conjugated drug (Conjugated NFV); the reaction start with the addition of specific anti-Nelfinavir antibodies. After one hour incubation, samples and reagents in excess are washed away.

The detection of the binding between the enzyme-conjugated drug and the specific antibody is performed by adding a chromogenic substrate.

The enzymatic activity produces a coloured solution, whose absorbance can be read by a microplate reader; the absorbance value is inversely correlated to the drug concentration in the sample.

3 TEST PERFORMANCES

The system is designed for the quantitative detection of Nelfinavir and its major metabolite M8 in human plasma.

- Dynamic range is from 0.8 to 3 micrograms of Nelfinavir and M8 per millilitre of plasma.
- Inter-assay variability calculated on 2 samples of negative plasma spiked with known quantities of drug tested in 5 different sessions is shown in the following table (concentrations are in µg/ml).

Sample Conc.	Conc run 1	Conc run 2	Conc run 3	Conc run 4	Conc run 5	Mean conc.	Std.D ev.	C.V.
1.5	1.3	1.4	1.1	1	1.3	1.2	0.2	13.5
3	2.4	2.5	2	1.7	2.9	2.3	0.5	20.2

- Intra-assay variability calculated on 2 samples of negative plasma spiked with known quantities of drug tested in triplicate is shown in the following table (concentrations are in µg/ml).

Sample	O.D. 1	O.D. 2	O.D. 3	Mean O.D.	Std.Dev.	C.V.
1.5	1.5	1.4	1.6	1.5	0.1	6.7
3	2.7	2.4	2.5	2.5	0.2	6

4 LIMITATIONS

No significant cross-reactivity (greater than 0,01% as per cent ratio of the IC₅₀ of the drugs) with other protease inhibiting drugs commonly used in HIV-1 therapy has been identified. Tested drugs include Ritonavir, Amprenavir, Saquinavir, Indinavir and Lopinavir

5 MATERIALS SUPPLIED

Contents	Quantity
Antibody-coated microtiterplate	12x8 wells
Nelfinavir antiserum	1x 12ml
Nelfinavir conjugate	1x 10ml
Nelfinavir Calibrators/Standard curve	7x 0.3ml
TMB 10X	1x 3ml
Development Solution	1x 30ml
Washing Solution 10X	1x 100ml
Stop Solution	1x 7ml

6 COMPOSITION OF SUPPLIED MATERIALS

Antibody-coated Microtiterplate

12 x 8 well strips, coated with anti-rabbit IgG sheep antibodies, sealed under vacuum in a polyethylene pouch.

Nelfinavir Antiserum

One vial containing 12 ml of anti-Nelfinavir rabbit antibodies, in a buffered solution containing a preservative.

Nelfinavir Conjugate

One vial containing 10 ml of Nelfinavir-horseradish peroxidase conjugate, in a buffered solution containing a preservative.

Nelfinavir Calibrators/Standard Curve

7 vials, each containing 0.3 ml of Nelfinavir in organic solvent solution. Calibrator values (ng/ml) are indicated on vial labels. Calibrators are ready to use.

TMB 10X

One non-transparent vial containing 3 ml of 10X TMB buffered solution.

Development Solution

One vial containing 30 ml of a buffered solution.

Washing Solution 10X

One bottle containing 100 ml of a 10X buffered solution.

Stop Solution

One vial containing 7 ml of 1M sulphuric acid solution.

7 MATERIALS REQUIRED BUT NOT PROVIDED

- Calibrated EIA reader at 450 and 620nm (able to detect O.D. in a range between 0.05 – 3.0); proper software for quantitative ELISA test management is strongly recommended (4 parameters Logit-Log).
- Microplate Washer
- Microcentrifuge for Eppendorf 1,5 ml tubes (10.000 x g).
- Vortex Mixer
- Precision pipettes with suitable tips (volumes between 10 and 1000 μ l, \pm 5% accuracy)
- Multichannel pipette with 8 tips (volumes between 50 and 300 μ l, \pm 5% accuracy)
- Reservoir (disposable) for multichannel pipette
- 10 and 25 ml pipette and pipette-handler
- 1.5 ml Eppendorf tubes
- Glass cylinders (500 and 1000 ml)
- Timer (60 min. range)
- Test tube racks (for 50 and 1.5ml tubes)
- Filter paper and aluminium foil
- Disposable gloves
- Methanol (99%).
- Deionized water (MilliQ grade)

- Methanol (analytic grade) solution at 30% in deionized water.

8 STABILITY AND STORAGE

- The kits should be stored at 2-8°C and used before the expiry date printed on the label on the box.
- Delivery should be done at controlled temperature (2-8°C); exposure to temperatures of up to 30°C for a short period of time (less than 6 hours) doesn't affect device performances.
- Do not freeze.
- Once opened, the kit reagents should be used within 1 month and properly stored at 2-8°C.
- The 10X wash solution may show precipitated crystals; make sure to dissolve them before making the dilution. Diluted washing buffer should not be stored for more than 4 hours.
- Substrate should be freshly diluted and not reused.
- Samples should not be stored for more than 4 hours after dilution.

9 PREPARATION OF REAGENTS

9.1 Reagents for sample pre-treatment

- Prepare a 30% solution of methanol in MilliQ water; we recommend about 10 ml per 10 samples.

9.2 Reagents for ELISA testing

- Use an appropriate scheme to register the position of calibrators, blanks and samples on the microplate. Calibrators, blanks and samples must be tested in duplicate.
- Remove only the number of strips required for the day's testing and place the remainder at 2-8°C in the foil pouch, sealing it carefully with a tape. If the whole kit is not to be used in a single run, take out only the reagents needed and place the rest immediately back at 2-8°C
- Use sterile tips for the Calibrators.
- Keep all reagents at room temperature for 30 minutes before use.
- Prepare the required volume of washing solution 1X in the glass cylinder by diluting 1:10 the Washing Solution 10X with deionized water (Check that no crystal be visible in the 10X Washing Solution).

- Prepare the required volume of chromogen solution in a clean container by diluting 1:10 the TMB 10X with Development Solution.
Warning: dilute the substrate just before use (after washing the plate, at the end of the first incubation) and keep away from direct light.
- Always wear disposable gloves.
- Return unused reagents at 2-8°C as soon as possible.

10 COLLECTION, STORAGE AND PREPARATION OF SAMPLES

- The device is designed for the analysis of heat-inactivated (30 min at 56° C) human plasma.
- No patient preparation is required for blood collection.
- Blood samples should be collected by qualified personnel using approved aseptic venipuncture techniques.
- It is important to preserve the chemical and biological sample characteristics till the analysis is complete.
- Do not add preservatives to the samples.
Warning: sodium azide is a strong inhibitor of horseradish peroxidase and should NOT be used.
- Samples should be stored at 2-8°C and analysed within 24 hours. For prolonged storage, freeze samples and store frozen at -20°C.
- Avoid repeated freeze-thaw cycles. If samples have to be frozen it is advisable to make aliquotes of at least 150 µl.
- Always wear disposable gloves.
- Thaw samples carefully if stored at -20°C. Mix carefully using a Vortex mixer
- Transfer 100 µl of plasma and dilute them with 300 µl of methanol.
- Mix well on a Vortex mixer for 10-15 sec.
- Centrifuge 10 min. at 10,000 x g.
- Take 100 µl of clear supernatant and dilute with 150 µl of MilliQ water.
- Mix well on a Vortex mixer for 10-15 sec.
- Take 10 µl of the dilution above and add it to 590 µl of 30% methanol.
- Mix very well using a Vortex mixer for 10-15 sec and use 20 µl in duplicate for testing.

11 ASSAY PROCEDURE

- Transfer 20 µl of the final dilution of each samples and Calibrators (ready to use) into the appropriate wells.
- Change pipette tip between samples.

- Dispense 80 µl of Nelfinavir conjugate in all wells, except for the blank wells, using a multichannel pipette.
- Dispense 100 µl of Nelfinavir antiserum in all wells, except for the blank wells, using a multichannel pipette.
- Incubate for 60 mins. at room temperature (RT).
- Wash the wells 5 times with 350 µl/well of 1X Washing Solution.

IMPORTANT: the washing steps are extremely important for the precision and accuracy of the test. Using an automated washer, always check its efficiency and do the maintenance suggested by the manufacturer.

- Add 200 µl of diluted chromogen solution to each well using the multichannel pipette.
- Incubate for 30 min. at room temperature (RT) in the dark.
- Read the absorbance at 620 nm by a microplate reader.

- Add 50 µl of Stop Solution in each well using the multichannel pipette.

IMPORTANT: mix carefully with a rotating movement microplate to obtain a homogeneous yellow color in each well.

- Read the absorbance at 450 nm using a microplate reader and use the data for the to calculate the sample concentration.

Warning 1: if some absorbance values obtained are over the measurement range of the microplate reader, use the measurement data at 620 nm.

Warning 2: final absorbances should be read within 15 minutes from addition of Stop Solution.

- Use appropriate software to plot the standard curve and calculate the concentration of Nelfinavir in the samples
- The test is valid if the following parameters are met:
 - Mean absorbance value of 0 Calibrator higher than 0,8 O.D.
 - Mean absorbance value of Blank lower than 0,3 O.D.

12 CALCULATION OF RESULTS

- If calculations are made using ELISA software, use a 4-Parameters Logit-Log method.
- If calculations are made manually, for every well calculate B/B₀ value. B/B₀ is expressed as follows:

Mean absorbance of Calibrators or Samples X 100

Mean absorbance of 0 Calibrator

Using semi-log chart paper, assign concentration values of Calibrators (as printed on labels) to the X-axis and B/B_0 values to the Y-axis. Draw the standard curve and interpolate the sample concentration value on it.

Warning: calculated concentration values must be multiplied by the dilution factor of samples (x 600) to obtain concentration in plasma.

13 PRECAUTIONS

- For research use only.
- This device is designed to be used by properly trained laboratory staff.
- The device and its components must be employed in accordance with current legislation.
- Device does not need to be deactivated.
- Always wear disposable gloves when using the device.
- In case of ingestion or contact with the eyes, skin or mucosae, wash with plenty of water and consult a physician.
- Unless the all kit not to be used, remove only the number of strips being used for the day's testing and place the remainder in foil pouch with desiccant at 2-8°C.
- Take only the appropriate amount of the reagents provided, leaving the bulk in the original vial.
- For reagents to be dispensed by Multichannel pipette, increase the volume by 1 ml.

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Codice 3678

96 wells

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