

TDM-Elisa LPV (Lopinavir)

For in vitro diagnostic use

1 INTENDED USE

TDM-ELISA LPV (Lopinavir) is a colorimetric competitive enzyme-immunoassay for the determination of the drug "Lopinavir" in plasma samples; it is useful in managing patient therapy and monitoring compliance.

2 PRINCIPLE OF THE TEST

The ELISA Lopinavir test is based on the competition between the drug in the plasma sample and the drug conjugated with the detection enzyme; they compete for the same binding site of a specific polyclonal antibody against LPV. A species-specific solid phase (wells of a microplate) binds the specific antibodies.

A washing step is used to wash away all unspecifically-bound material from the solid phase. The binding between the enzyme-conjugated drug and the specific antibody is detected 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 Lopinavir in plasma samples.

Dynamic range is from 1 to 8 micrograms of drug per millilitre of plasma.

- The following table shows the ELISA / HPLC inaccuracy values calculated on 4 samples of plasma from patients on HAART with Lopinavir and 2 reference samples normally used for QC by HPLC labs:

Sample N°	ELISA µg/ml	HPLC µg/ml	Inacc. %
1	2,19	2,3	-4,78
2	4,17	5,6	-25,54
3	1	1,2	-16,67
4	1,86	1,7	9,41
CQ-A	4,69	4,81	-2,5
CQ-B	2,75	2,83	-2,9

- Intra-assay variability calculated on n.3 samples of negative plasma spiked with known quantities of drug tested in quadruplicate in the same run is shown in the following table (concentrations are in µg/ml). Nominal values of expected concentration (spike) are shown in the first column. The values of the 2 reference samples of the table above are also reported with reference to HPLC analysis;

LPV (µg/ml)	Valore 1 ug/ml	Valore 2 ug/ml	Valore 3 ug/ml	Valore 4 ug/ml	Media ug/ml	DEV STD	CV
5	4,38	4,3	3,75	4,75	4,30	0,41	9,61
2,5	2,53	2	2	2,25	2,20	0,25	11,50
1,25	1,4	1,1	1,25	1,25	1,25	0,12	9,80
CQ-A (4,81)	5,125	4,25	-	-	4,69	0,62	13,20
CQ-B (2,83)	3	2,5	-	-	2,75	0,35	12,86

In the following table inter-assay precision is reported. 3 negative plasma samples to which known quantities of Lopinavir were added have been tested in several runs. Expected "spike" values are shown in the first column. Values from 3 samples of patients on HAART with Lopinavir are also reported:

LPV (µg/ml)	1 µg/ml	2 µg/ml	3 µg/ml	4 µg/ml	5 µg/ml	Media µg/ml	DEV STD	CV
5	3,87	4,65	4,02	4,52	5	4,41	0,47	10,63
2,5	2,53	2,69	2,04	2,27	2,2	2,34	0,27	11,69
1,25	1,38	1,2	0,92	1,128	1,1	1,16	0,14	12,19
#1	9,3	7,785	-	-	-	8,54	1,07	12,5
#2	9,25	10,64	-	-	-	9,95	0,98	9,9
#3	1,37	1,148	-	-	-	1,26	0,16	12,5

4 SPECIFICITY AND LIMITATIONS

- No significant cross-reactivity (greater than 0.01% as ratio of the drugs IC₅₀) with other protease inhibiting drugs commonly used in HIV-1 therapy was identified. Tested drugs include Ritonavir, Amprenavir, Saquinavir, Indinavir and Nelfinavir at therapeutic concentrations.

5 MATERIALS SUPPLIED

Microplate with absorbed antibodies	12 x 8 wells
LPV antiserum	1x 12ml
Enzyme -LPV	1x 10ml
Lopinavir calibrators/ standsrd curve	6 x 150 µl
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.

Anti-LPV antiserum

One vial containing 12 ml of anti-Lopinavir rabbit antibodies, in phosphate buffer 10 mM pH 7.4 + 0.3% gelatin + sodium azide 0.01%.

LPV-horseradish peroxidase conjugate

One vial containing 10 ml of Lopinavir-horseradish peroxidase conjugate, in phosphate buffer 10 mM pH 7.4 + 0.3% gelatin + sodium azide 0.01%.

LPV calibrators/ Standard curve

6 vials, each containing 150 µl of Lopinavir in methanol 30%. Calibrator values are indicated on vial labels. Calibrators are ready to use.

Caution: this solution is classified as toxic.

TMB 10X

One non-transparent vial containing 3 ml of tetrametilbenzidine 1mg/ml

Development Solution

One vial containing 30 ml of citrate buffer 0.1M pH 4 + H₂O₂ 0.01%.

Washing Solution 10X

One bottle containing 100 ml of a phosphate buffer 100 mM pH 7.4 + Tween 20 0.5%.

Stop Solution

One vial containing 7 ml of 1.38 M sulphuric acid solution.

Caution: this solution is considered irritant.

7 MATERIALS REQUIRED BUT NOT PROVIDED

- Calibrated EIA reader adjusted to read at 450 and 620nm (able to detect O.D. in a range between 0.05 – 3.0 O.D.); proper software for quantitative ELISA test management is highly recommended.
- Microplate Washer
- Precision pipettes with suitable tips (volumes between 20 and 1000µl, ± 5% accuracy)
- Multichannel pipettes (volumes between 50 and 300 µl, ± 5% accuracy)
- Methanol analytical grade (99%)
- Methanol solution at 30%
- Deionized water (MILLI Q grade)
- Vortex
- Microcentrifuge for 1.5ml Eppendorf tubes (10.000 x g)
- 1.5ml Eppendorf tubes
- Reservoir (disposable) for multichannel pipette
- Glass cylinder (1000 ml)
- 10 ml and 25 ml pipette and pipette-holder
- Timer (range 60 min.)
- Test tube racks (for 50 and 1.5 ml tubes)
- Filter paper and aluminium foil
- Disposable gloves

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.
- Controlled temperature (2-8°C) should be maintained during delivery; however 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.
- Expiry date is printed on the label on the box.
- Once opened, the kit reagents should be used within 1 month and properly stored at 2-8°C and.
- Diluted washing buffer should not be stored for more than 4 hours and must be discarded after use.
- Substrate should be freshly diluted and not reused.
- Samples should not be stored for more than 4 hours after dilution.

9 PREPARATION OF REAGENTS/SAMPLES

9.1 Reagents for sample pre-treatment

- Prepare a 30% solution of methanol in MilliQ deionized water employing about 20 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.
- If the entire kit is not to be used at once, remove only the number of strips required for the day's testing and place the remainder at 2-8°C in foil pouch, sealing it carefully with a tape.
- Use sterile tips for the Calibrators.
- Keep all reagents at room temperature for 30 minutes before use.
- Prepare the required volume of 1X washing solution by diluting in the ratio 1:10 the 10X Washing Solution with deionized water in a clean glass cylinder.
- Prepare the required volume of chromogen solution by diluting in the ratio 1:10 the 10X TMB with the Development Solution in a clean container.

Warning: dilute the substrate just before use (after washing the plate) and keep away from direct light.

- Always wear disposable gloves.
- Return unused reagents at 2-8°C as soon as possible.

9.3 Preparation of samples

- The device is designed for the analysis 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 characteristics of the samples until 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.
- Always wear disposable gloves.
- Thaw samples carefully if stored at -20°C. Mix carefully using a Vortex mixer for 10-15 sec.
- Transfer 100 µl of each sample in a tube and dilute with 300 µl of methanol (99%). Mix carefully using a Vortex mixer for 10-15sec.
- Centrifuge for 10 min. at 10.000 x g.
- Take 100 µl of clear supernatant and dilute it with 150 µl of deionized water. Mix well, using a vortex mixer for 10-15 sec.
- Take 100 µl of the dilution above and add it to 150 µl of a 30% methanol solution. *Mix very well using a Vortex mixer for 10-1 sec. and use 20 µl for testing.*

10 ASSAY PROCEDURE

- Transfer 20 µl of the final dilution of each sample and Calibrators (ready to use) into the appropriate wells.
- Change pipette tip between samples.
- Dispense 80 µl of LPV-horseradish peroxidase conjugate in all wells, except for the blank wells, using a multichannel pipette.
- Dispense 100 µl of Anti-LPV rabbit 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 1X Washing Solution using 350 µl/well.
- 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 using a microplate reader.
- Add 50 µl of Stop Solution in each well using the multichannel pipette.
- 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 at 450nm 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 Lopinavir 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.

11 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/B₀ values to the Y-axis. Draw the standard curve and interpolate the sample concentration value on it .

12 PRECAUTIONS

- 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.
- If all the kit is not to be used at one time, remove only the number of strips needed 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.

TDM LPV (ELISA Lopinavir)

96 wells, code 2678

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Fabbricante:

BioStrands S.r.l.,
Via del Follatoio 12
34148 Trieste (Italia)
Tel. +39.040.8992.451

