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## IMUBIND® 12-Lipoxygenase ELISA

Product No. 872

Storage: 2–8°C

For Research Use Only!

### INTENDED USE

The IMUBIND® 12-Lipoxygenase (Platelet-type/arachidonate, 12-LOX, 12S-LOX, ALOX12) ELISA is an enzyme-linked immunosorbent assay for the quantitative determination of human 12-Lipoxygenase in human plasma or serum or in any fluid where 12-Lipoxygenase might be present.

### EXPLANATION OF THE TEST

Human 12-Lipoxygenase is physiologically expressed in cells of the megakaryocytic lineage, in umbilical vein endothelium, and in epidermal cells. Its unique functions are the oxygenase and lipoxin synthase activities. 12-Lipoxygenase is localized to the cytoplasm.

### Relevance

- 12-LOX is overexpressed in different types of cancers and plays an important role in cancer pathophysiology.
- Platelet-type 12-LOX is expressed in human melanoma cells of different origin, in their transplanted xenografts, and in fresh human skin tumors.
- Platelet-type 12-LOX is expressed in spontaneously metastasizing xenografts and in thick human skin tumors.
- Platelet 12-lipoxygenase (P-12-LOX) expression is elevated in prostate cancer and the level of expression is correlated with the grade of this cancer.
- Platelet-type 12-Lipoxygenase (12-LOX) has been shown to regulate growth, metastasis, and angiogenesis of prostate cancer.

### PRINCIPLE OF THE METHOD

Diluted samples are added to microwells coated with a polyclonal antibody against 12-Lipoxygenase. During an incubation period, 12-Lipoxygenase present in the sample will bind to the antibody coated to the wells. Following a washing step, a horseradish peroxidase (HRP) conjugated anti-12-Lipoxygenase monoclonal antibody is added to the microwells and binds to the 12-Lipoxygenase protein captured on the plate during a short incubation period. Following another washing step, the addition of a perborate-3,3'-5,5'-tetramethylbenzidine (TMB) substrate and its subsequent reaction with the HRP present generates a blue colored solution. The reaction is stopped by adding citrate stop solution, which turns the solution color yellow. Measuring the solution absorbance at 450 nm and extrapolating the value with those of a standard curve determines the level of 12-Lipoxygenase in the diluted sample.

### REAGENTS

- R1** Antibody Coated Microtiter plate, MTP-96 (12x8) well
- R2** Wash buffer, 50 ml, 1 vial (concentrate)
- R3** Dilution buffer, 50 ml, 1 vial (ready-to-use)
- R4** 12-LOX Standard, 25 ng/ml, 0.5 ml, 2 vials (lyophilized)
- R5** Antibody conjugate, HRP-conjugated anti-human 12-LOX, 11 ml, 1 vial (ready-to-use)
- R6** Substrate, 11 ml, 1 vial (ready-to-use)
- R7** Stop solution, 6 ml, 1 vial (ready-to-use)

### REAGENT PREPARATION AND STORAGE

Unopened and lyophilized reagents are stable until the expiration date printed on the box when stored as instructed.

- R1 Antibody coated microwells:** Once removed from the foil pouch, the microwell strips must be used within 30 minutes. Unused strips may be stored at 2°-8°C for 4 weeks when sealed in the original pouch with the desiccant present, protected from any moisture.
- R2 Wash buffer:** Transfer the content to a 1 liter bottle and fill up the concentrate to 1 liter with filtered deionized/distilled water. Diluted Wash Buffer may be used for up to 4 weeks when stored at 2°-8°C.
- R3 Dilution buffer:** Supplied ready to use. Opened dilution buffer is stable for 3 month when stored at 2°-8°C.
- R4 Standard:** Reconstitute the standard with 500 µl purified, deionised or distilled water, swirl the contents gently and allow the vials to stand at room temperature for at least 15 minutes to ensure complete dissolution. The lyophilised standard plasma is stable until the date indicated on the vial label when stored at 2° - 8°C. Once reconstituted, the standard will remain stable for 3 month when stored at -20 °C.
- R5 Antibody conjugate:** Supplied ready to use. Opened conjugate is stable for 3 month when stored in the dark at 2°-8°C.
- R6 Substrate, TMB:** Supplied ready to use. Opened substrate is stable for 3 month when stored in the dark at 2° - 8°C.
- R7 Stop solution:** Supplied ready to use. Opened stop solution is stable for 3 month when stored at 2° - 8°C.

### SPECIMEN COLLECTION AND PREPARATION

Citrate collected **platelet rich plasma** (PRP) may be used for this assay. Do Not Use EDTA. See "Collection, Transport and Processing of Blood Specimens for Testing Plasma-based Coagulation Assays; Approved Guidelines-Fourth Edition", NCCLS Document H21-A4, Vol. 23, No. 35, December 2003. Plasma collection should be performed as follows:

1. Collect 9 parts of blood into 1 part of 3.2% (0.109 M) trisodium citrate anticoagulant solution.
2. Centrifuge the blood sample at **150 x g for 15 minutes**.
3. Plasma should be stored at 2°-8°C and assayed within 4 hours. Alternatively, plasma may be stored at -20°C for up to 6 months.
4. Frozen plasma should be thawed rapidly at 37°C. Thawed plasmas should be stored at 2°-8°C and assayed within 4 hours.

### PROCEDURE

#### Materials Provided – See Reagents

#### Material Required But Not Provided

0.22 µm filtered deionized H<sub>2</sub>O  
50-300 µL eight channel multi-pipette  
0-200 µL, 200-1000 µL single pipettes  
microwell plate reader for reading absorbance at 450 nm  
microwell plate washer (optional), microwell plate shaker (optional)

#### Assay Procedure

Running standard in duplicate is recommended.

#### Preparing the 12-Lipoxygenase Standards

1. Open the foil pouch and remove the microwell strips/frame assembly. Remove the strips that will not be used, return them to the foil pouch and tightly reseal the pouch with the desiccant inside. Store the foil pouch at 2 - 8°C.
2. Reconstitute the standard as instructed under REAGENT PREPARATION. Prepare five (6) serial dilutions of the standard as follows: Add 200 µL of the standard to microwells A1/A2. Add 100 µL of dilution buffer to microwells B1/B2-G1/F2. Serially dilute the standard by pipetting 100 µL of the standard from microwells A1/A2 into microwells B1/B2. Mix and pipette 100 µL from wells B1/B2 to wells C1/C2. Repeat this process through wells G1/G2. Remove and discard 100 µL from wells G1/G2. The concentrations of the serially diluted standard will be 25, 12.5, 6.25, 3.12, 1.56, 0.78, and 0.39 ng/mL respectively. For a standard of 0 ng/mL, add 100 µL of dilution buffer to microwells H1/H2.

## Sample preparation

### Recommendation for platelet rich plasma:

3. Dilute each plasma sample 1:100 with dilution buffer (**other samples** have not been tested yet, the optimal dilution has to be determined).
4. Pipette 100  $\mu$ L of the diluted samples into separate microwells, cover with the acetate sheet and incubate for 1 hour at room temperature (18-25°C) on an orbital microwell plate shaker with agitation (at 250 rpm).
5. Empty the contents of the microwells and wash 4 times with Wash Buffer. Washing may be performed either using microwell plate washing equipment or manually (fill the wells with Wash Buffer with a pipette or squeeze bottle, wait three minutes, empty and remove droplets by tapping the plate 4-5 times face down against absorbing material).
6. Add 100  $\mu$ L of Detection Antibody to each microwell, cover with the acetate sheet and incubate the wells for 1 hour at room temperature (18-25°C) on an orbital microwell plate shaker with agitation (at 250 rpm).
7. Wash the wells by repeating Step 5.
8. Add 100  $\mu$ L of Substrate to each microwell immediately after the wash step, cover the wells with the acetate sheet and incubate for 5-10 minutes at room temperature (18°-25°C). A blue color will develop.
9. Stop the enzymatic reaction by adding 50  $\mu$ L Stop solution to each microwell. Add the acid with the same speed and order as you added the substrate. Tap the sides of the microwell frame to ensure even distribution of the solution. The solution color will turn yellow. Read the absorbance on a microwell plate reader at a wavelength of 450 nm within 10 minutes.

## RESULTS

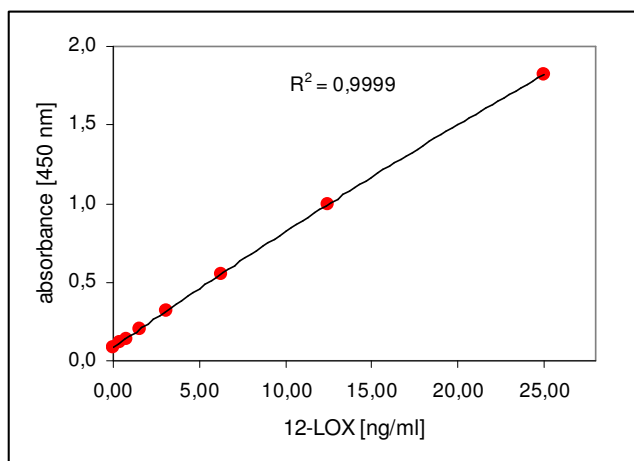
Construct a standard curve by plotting the mean absorbance value for each standard versus its corresponding concentration. A standard curve should be generated each time the assay is performed. The following standard curve is for demonstration purposes only.

## CALCULATIONS

Determine the amount of 12-Lipoxygenase in the diluted sample by interpolating directly from the standard curve. If the sample was diluted 1:100 during its preparation, multiply the results by 100 in order to obtain the concentration of 12-Lipoxygenase in the neat plasma sample. The calculation is:

$$[12\text{-Lipoxygenase}]_{\text{Sample}} = [12\text{-Lipoxygenase}]_{\text{Diluted Test Sample}} \times 100$$

## Representative Standard Curve



## PERFORMANCE CHARACTERISTICS

### Specificity

The antibodies are specific for 12-LOX/ALOX12. No cross-reactivity was observed against other members of the Lipoxygenase family (5-LOX, 15-LOX-1, 15-LOX-2, 12R-LOX, and eLOX) when tested in ELISA and native PAGE.

### BIBLIOGRAPHY

- Human platelet 12-lipoxygenase, new findings about its activity, membrane binding and low-resolution structure. Aleem AM, Jankun J, Dignam JD, Walther M, Kühn H, Svergun DI, Skrzypczak-Jankun E. *J Mol Biol.* 2008 Feb 8;376(1):193-209
- Vascular endothelial growth factor production in human prostate cancer cells is stimulated by overexpression of platelet 12-lipoxygenase. McCabe NP, Selman SH, Jankun J. *Prostate.* 2006 May 15;66(7):779-787.
- Expression of cyclooxygenase-2 and 12-lipoxygenase in human breast cancer and their relationship with HER-2/neu and hormonal receptors: impact on prognosis and therapy. Mohammad AM, Abdel HA, Abdel W, Ahmed AM, Wael T, Eiman G. *Indian J Cancer.* 2006 Oct-Dec;43(4):163-168.
- Synthetic curcuminoids modulate the arachidonic acid metabolism of human platelet 12-lipoxygenase and reduce sprout formation of human endothelial cells. Jankun J, Aleem AM, Malgorzewicz S, Szkudlarek M, Zawodzky MI, Dewitt DL, Feig M, Selman SH, Skrzypczak-Jankun E. *Mol Cancer Ther.* 2006 May;5(5):1371-1382.
- Molecular identification, localization and function of platelet-type 12-lipoxygenase in human melanoma progression, under experimental and clinical conditions. Rásó E, Döme B, Somlai B, Zacharek A, Hagmann W, Honn KV, Tímár J. *Melanoma Res.* 2004 Aug;14(4):245-50.
- Platelet-type 12-lipoxygenase activates NF-kappaB in prostate cancer cells. Kandouz M, Nie D, Pidgeon GP, Krishnamoorthy S, Maddipati KR, Honn KV. *Prostaglandins Other Lipid Mediat.* 2003 Jul;71(3-4):189-204.
- Expression of 12-lipoxygenase as a biomarker for melanoma carcinogenesis. Winer I, Normolle DP, Shureiqi I, Sondak VK, Johnson T, Su L, Brenner DE. *Melanoma Res.* 2002 Oct;12(5):429-34.
- Expression, subcellular localization and putative function of platelet-type 12-lipoxygenase in human prostate cancer cell lines of different metastatic potential. Tímár J, Rásó E, Döme B, Li L, Grignon D, Nie D, Honn KV, Hagmann W. *Int J Cancer.* 2000 Jul 1;87(1):37-43.