IMMBIOMED

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IMUBIND® 12-Lipoxygenase ELISA

Product No. ADG872

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-a **Detection antibody**, biotinylated anti-human 12-LOX mAb, 120 µl, 1 vial (100x concentrate)
- R5-b **Conjugate buffer**, 25 ml, 1 vial (ready-to-use)
- R5-c **Enzyme conjugate**, SA-HRP, 120 µL (100x concentrate)
- 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 10 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-b **Conjugate buffer:** Supplied ready to use. Opened dilution buffer is stable for 3 month when stored at 2°-8°C.

R5-a **Detection antibody:** Supplied as a concentrate, dilute the Detection antibody 1:100 with Conjugate bBuffer just prior to use. For using all 96 microwells at one time, dilute 100 µL of Detection antibody to 10 mL in Conjugate buffer. If not all 96 microwells are used, dilute 10 µL of Detection antibody to 1 mL in Conjugate buffer for each 8-microwell strip that will be used. Working strength Detection antibody is stable for 4 hours at 2°-8°C. Discard any unused working strength Detection antibody. Opened antibody is stable for 3 month when stored in the dark at 2°-8°C.

R5-c **Enzyme conjugate:** Supplied as a concentrate, dilute the Enzyme conjugate 1:100 with Conjugate buffer just prior to use. For running all 96 microwells at one time, dilute 100 µL of Enzyme conjugate to 10 mL in Conjugate buffer. If not all 96 microwells are used, dilute 10 µL of Enzyme conjugate to 1 mL in Conjugate buffer for each 8-micro-well strip that will be used. Working strength Enzyme conjugate is stable for 2 hours at 2°-8°C. Discard any unused working strength Enzyme conjugate.

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_2O
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)

Preparing the 12-Lipoxygenase Standards

1. Reconstitute the 12-LOX standard as instructed under REAGENT PREPARATION.
2. Prepare 7 serial dilutions of the reconstituted standard with Dilution buffer as follows. Use Dilution buffer as the 0% standard.

Tube		Dilution buffer	Conc. [ng/ml]
(1)	200 µl reconstituted standard	300 µl	10
(2)	200 µl from (1)	300 µl	4.0
(3)	200 µl from (2)	300 µl	1.6
(4)	200 µl from (3)	300 µl	0.64
(5)	200 µl from (4)	300 µl	0.26
(6)	200 µl from (5)	300 µl	0.10
(7)	200 µl from (6)	300 µl	0.04
(8)	-	300 µl	0

Preparing the Sample Dilutions

3. Recommendation for **platelet rich plasma**: Dilute each plasma sample 1:100 with dilution buffer.

   Recommendation for **tissue extracts:** Dilute each tissue extract 1:5 with dilution buffer.

   (Other samples have not been tested yet, the optimal dilution has to be determined).

Assay Procedure

4. Open the foil pouch and remove the microwell plate. 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.
5. Pipette 100 µL 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 µL working strength 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 µL working strength Enzyme Conjugate 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).
9. Wash the wells by repeating Step 5.
10. Add 100 µL of Substrate to each microwell immediately after the wash step, cover the wells with the acetate sheet and incubate for 20-25 minutes at room temperature (18°-25°C). A blue color will develop.
11. Stop the enzymatic reaction by adding 50 µL 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.

Hinweis/Note:

Der Packungsbeileger dient nur als erste Information. Der relevante Packungsbeileger liegt der Ware bei.

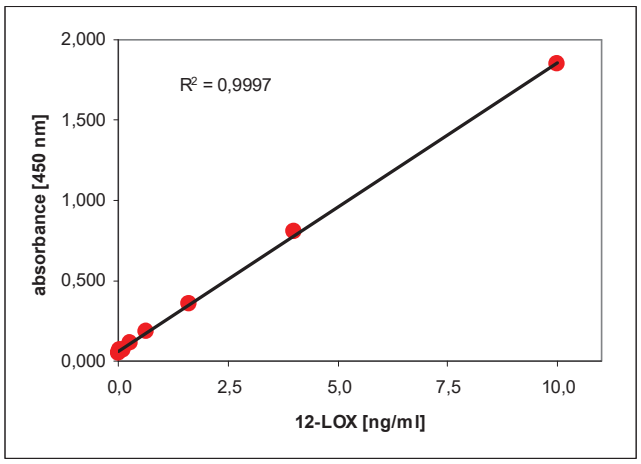
The datasheet is for information purposes only. The current datasheet will be enclosed with product shipment.

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:

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

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.

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