FluoBolt™-WNT3A

METAL ENHANCED FLUORESCENCE IMMUNOASSAY for human WNT3A

METAL ENHANCED FLUORESCENCE IMMUNOASSAY FOR
THE QUANTITATIVE DETERMINATION OF
WNT3A IN HUMAN SERUM AND PLASMA

CAT. NO. FIA-1705-F,-C3,-C5,-A6
96 Well Formate

CE

rev.no. 200312

FIANOSTICS GmbH, A-2700 Wiener Neustadt, Viktor Kaplan Strasse 2

Tel. + 43/2622/27514, E-mail office@fianostics.at

CONTENT

FIANOSTICS
Fluorescence Immunoassays

www.fianostics.at

1) METAL / PLASMON ENHANCED FLUORESCENCE

Metal Enhanced Fluorescence (MEF) offers the possibility to increase the analytical sensitivity of systems based on fluorescence detection dramatically. MEF is based on the fact that excitation light interacts with the electrons of metal nano-structures thus generating very high electromagnetic fields (Localised Surface Plasmons, LSPs) Therefore, such structures are also called "plasmonic structures" and the combination of (e.g. polymeric) support and structure is known as "plasmonic substrate". These LSPs lead to an increase in emission output of fluorescent molecules (e.g. fluorescently labeled antibodies) when bound to surfaces with suitable nano-metal structures that enhances the signal dramatically. FIANOSTICS has developed a new plasmonic enhanced immunoassay platform in cooperation with Sony DADC BioSciences (now STRATEC Consumables since July 1st 2016), that allows up to 300 fold gains of sensitivity. This platform is fully compatible to standard laboratory methodology using 96 well microtiter plate format and assays based on this technology can be run on any standard fluorescence microplate reader. Its unique features enable fluorescence immunoassays with highest sensitivity and without washing steps.

2) WNT3A

WNT3A is a secreted glycoprotein and belongs to the WNT family. Members of this family can interact with cell membrane receptors, thus playing a role in autocrine regulations and paracrine signaling. WNT3A is expressed in placenta at moderate levels, as well as in lung, spleen and prostate at low levels. The canonical sequence of WNT3A consists of 352 amino acids (aa) and has a mass of 39.365 kDa. It is rich in cysteine and forms many disulfide bonds from cysteine residues. At aa 87 and aa 298 glycosylation appears, since *N*-Acetylglucosamine is covalently attached to asparagine. At position aa 209 WNT3A is

covalently lipidated at a conserved serine residue resulting in strong hydrophobic properties of the molecule. Therefore, in its physiological form it constitutes a soluble complex with afamin, which functions as a carrier for hydrophobic molecules in body fluids and is essential for the activity and solubility of WNT3A.

WNT3A plays important roles in cell growth and differentiation, embryonic development, neural development, immune regulation, bone formation and carcinogenesis. Therefore, research has investigated association of elevated expression of WNT3A with prostate, breast or hepatocellular cancer.

3) CONTENT OF THE KIT

ID	KIT COMPONENT	QUANTITY
GM	Anti-human WNT3A antibody, pre-coated MEF-microtiter plate, packed in vacuum sealed aluminum bag	1 x 96 well
WP	Wash buffer concentrate 20x, natural cap	1 x 25 ml
GAF, GA3, GA5, GAA	Anti-human WNT3A antibody, labeled with FITC, Cy3, Cy5 or AlexaFluor680, black flask	1 x 2.5 ml
GS	Standards 1-6, (2800, 1400, 700, 350, 175, 0 pmol/l), white caps, lyophilized	6 vials, 0.25 ml
GCA/B	Control A and B, yellow cap, lyophilized (for concentrations see label)	2 vials, 0.25 ml
GD	Sample diluent, natural cap, ready to use	1 x 10 ml

4) ADDITIONAL MATERIAL SUPPLIED WITH THE KIT

- 2 self-adhesive plastic films
- QC data sheet
- Protocol sheet

- Instruction manual for use
- 2 desiccant bags for plate storage

5) MATERIAL AND EQUIPMENT REQUIRED BUT NOT SUPPLIED

- Precision pipettes calibrated to deliver 10 µl, 20 µl, 50 µl, 200 µl, 500 µl and disposable tips
- Distilled or deionized water
- Plate washer, multichannel pipette or manifold dispenser for washing
- Refrigerator with 4°C (2-8°C)
- Fluorescence microplate reader
- Graph paper or software for calculation of results

6) REAGENTS AND SAMPLE PREPARATION

All reagents of the kit are stable at 4°C (2-8°C) until expiry date stated on the label of each reagent.

Sample preparation:
Collect venous blood samples by using standardized blood collection tubes for serum or plasma. We recommend performing plasma or serum separation by centrifugation as soon as possible, e.g. 10 min at 2000 x g, preferably at 4°C (2-8°C). The acquired plasma or serum samples should be measured as soon as possible. Since WNT3A is not stable at room temperature, samples should not be stored at room temperature for longer periods (> 1 hour). For longer storage aliquot samples and store at -25°C or lower. Do not freeze-thaw samples more than 5 times.

Lipemic or hemolyzed samples may give erroneous results. Samples should be mixed well before assaying.
For further information on sample stability contact us by e-mail at support@fianostics.at or by phone + 43/2622/27514.

Reagent preparation:
Add 250 µl of distilled or deionized water to the lyophilized GS (Standards) and GC (Controls). Leave at room temperature (18-26°C) for at least 15 min but maximum 30 min before use in the assay. Since WNT3A is not stable at room temperature, reconstituted standards and controls should not be stored at room temperature for longer periods (> 1 hour). Reconstituted GS and GC are stable at -25°C or lower until expiry date stated on the label. Reconstituted GS and GC can undergo up to 5 freeze-thaw cycles.

Bring WP (Wash buffer) concentrate (20x) to room temperature. Make sure that the solution is clear and without any salt precipitates before further dilution. Dilute the WP to working strength by adding the appropriate amount of distilled or deionized water, e.g. 25 ml of WP + 475 ml water, prior to use in the assay. Undiluted WP is stable at 4°C (2-8°C) until expiry date on the label. Diluted WP is stable at 4°C (2-8°C) up to one month. Only use diluted WP in the assay.

7) ASSAY PROCEDURE

All reagents and samples must be at room temperature (18-26°C) before use in the assay.

Mark position for standards, controls and samples on the protocol sheet. We recommend running samples and standards in duplicates.

Take the plasmonic enhanced microtiter plate out of the aluminum bag. Avoid touching the bottom of the plate with bare hands, because reading without washing is performed through the well bottom.

Seal all wells that **will not be used** in the following assay run with the accompanying adhesive film (cut to fit!).

In standard format, the kit is delivered with an AlexaFluor680 labeled

detection antibody (GAA) since serum background fluorescence is minimal within this wavelength range. Therefore, if your reader is equipped with monochromatic optics, please set Excitation/Emission to 679/702 nm or if you are using an optical filter-based reader, select a suitable filter pair (e.g. 670/720 nm). On request the kit can also be delivered with FITC, Cy3 or Cy5 (Ex/Em = 495/518 nm, 550/570 nm or 650/670 nm) labeled antibody.

1) Add 25 µl of labeled detection antibody (GA) to all wells required. Swirl gently.

2) Add 20 µl of standard, control or sample to the wells according to the marked positions on the protocol sheet, swirl gently, cover tightly with the delivered adhesive film and incubate over night at room temperature (18-26°C) in the dark.

3) The next day read the plate either without any further processing (3a) or after performing a washing step (3b) using a fluorescent microplate reader.

Hint: Quality of bottom reading (3a) may vary between microplate readers. For first time users we suggest performing the washing step and follow protocol (3b).

3a) If your reader allows bottom reading, read the plate without any further processing at the Ex/Em wavelength fitting to the delivered antibody (495/518 nm for GAF, 550/570 nm for GA3, 650/670 nm for GA5, 679/702 nm for GAA). Gain should be set to achieve at least 10000 fluorescence units (F.U.) between the signal of the 0 pmol/l and the 2800 pmol/l WNT3A standard. Samples with signals exceeding the signal of the highest standard must be re-run with an appropriate dilution using sample diluent (GD).

3b) If your reader has no bottom read option or if you want to store the plate for documentation purposes, discard or aspirate the content of the wells and wash 3x with diluted wash buffer. Use a minimum of 200 µl wash buffer per well. After the final wash, remove remaining fluid by strongly tapping the plate against a paper towel. Read the plate in top configuration without any further processing at the Ex/Em wavelength fitting to the chosen antibody (495/518 nm for GAF, 550/570nm for GA3, 650/670 nm for GA5, 679/702 nm for GAA).

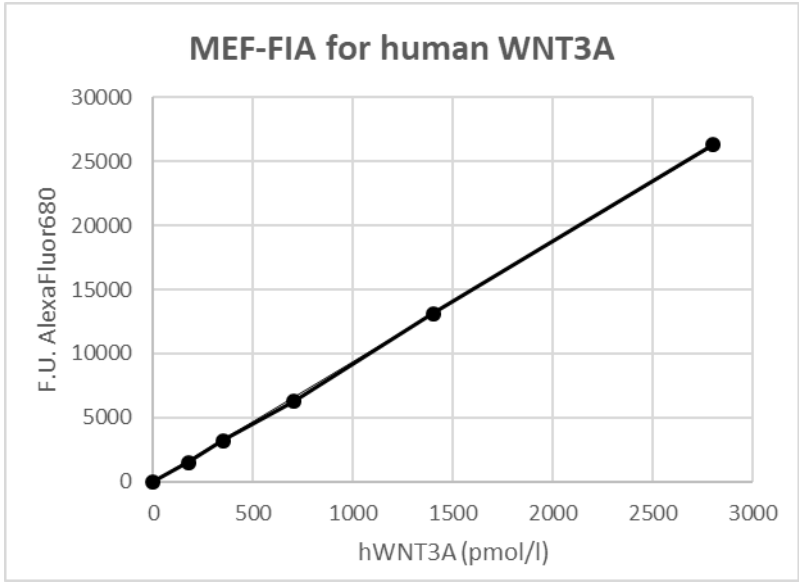
Gain should be set to achieve at least 10000 fluorescence units (F.U.) between the signals of the 0 pmol/l and the 2800 pmol/l WNT3A standard. Samples with signals exceeding the signal of the highest standard must be re-run with appropriate dilution using sample diluent (GD).

5) Store the plate with the 2 desiccant bags supplied at 4°C (2-8°C) in the aluminum bag. Unused wells are stable until expiry date stated on the label. Fluorescence signals of standards, controls and samples remain detectable for at least two months at the plate surface, depending on signal intensity achieved.

8) CALCULATION OF RESULTS

Subtract the fluorescence intensity of the 0 pmol/l standard from all other standards, samples and controls. Construct a calibration curve from the fluorescence units (F.U.) of the standards using commercially available software or graph paper. Read sample and control concentrations from this standard curve. Make sure to use appropriate curve fitting algorithm (e.g. linear or 4PL).

Example of a typical calibration curve:



The quality control (QC) protocol supplied with the kit shows the results of the final release QC for each kit lot at production date.

Fluorescence intensity obtained by customers may differ due to various influences and/or due to the normal decrease of signal intensity during shelf life.

However, this does not affect validity of results as long as the supplied kit controls read according to specifications (target ranges see labels).

9) ASSAY CHARACTERISTICS

Method	Metal Enhanced Direct Sandwich Fluorescence Immunoassay in 96-well plate format
Sample type	Serum, Plasma
Standard range	0 to 2800 pmol/l (6 standards and 2 controls in a serum-based matrix)
Conversion factor	1 ng/ml = 25 pmol/l (MW: 39.4 kDa)
Sample volume	20 µl (undiluted sample) / well
Incubation time / temperature	overnight / room temperature (18-26°C)
Sensitivity	LOD (0 pmol/l + 3 SD): 51 pmol/l; LLOQ: 175 pmol/l
Specificity	This assay detects human WNT3A
Cross-reactivity	Human WNT3A shares around 100-97% aa sequence with primates, 96-95% bears, 96% whales and 96% mice. Cross-reactivity of this assay with other species than human has not been tested.

Precision:

Intra-assay: 4 samples of known concentrations were tested 3 times within 1 assay run
CVs ranged from 3-10%.

Inter-assay: 4 samples of known concentrations were tested in duplicates within 3 different assay runs
CVs ranged from 7-11%.

Spike/Recovery:

The recovery of WNT3A in serum was evaluated by adding known amounts of human recombinant WNT3A to 4 different human serum samples. Mean recovery was 87%

Mean recovery in plasma was significantly lower than in serum:
Citrate-plasma: 45% (n=6)
EDTA-Plasma: 58% (n=8).

Linearity:

3 human serum samples were spiked with recombinant WNT3A and diluted 1+1 and 1+2 with the sample diluent (GD) supplied with the kit. Mean linearity was 74%.

Specificity:

Analyte Specificity:
This assay detects human WNT3A

Species Specificity:
Human WNT3A shares around 100-97% aa sequence with primates (e.g. orangutan or chimpanzee), 96-95% bears (e.g. grizzly bear or giant panda), 96% whales (e.g. beluga whale or dolphins) and 96% mice. Reactivity of this assay with other species than human has not been tested. So, using this assay for WNT3A measurements in serum or plasma of species with high sequence homology may be possible but must be evaluated by the user. FIANOSTICS does not take responsibility for functionality of the assay in non-human samples

10) TECHNICAL HINTS

- Do not mix or substitute reagents with those from other lots or sources.
- Do not mix stoppers and caps from different reagents or use

- reagents between lots.
- Do not use reagents beyond expiration date.
- Protect reagents from direct sunlight.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Avoid foaming when mixing reagents

11) PRECAUTIONS

- All test components of human source were tested against HIV-Ab and HBsAg and were found negative. Nevertheless, they should be handled and disposed as if they were infectious.
- Liquid reagents contain ≤0.1% Proclin 300 as preservative. Proclin 300 is not toxic in concentrations used in this kit. It may cause allergic skin reactions – avoid contact with skin, eyes or mucous membranes.
- Do not pipette by mouth.
- Do not eat, drink, smoke or apply cosmetics where reagents are used.
- Wear gloves, protective glasses and lab jacket while performing this assay.

12) LITERATURE

- **Single step, direct fluorescence immunoassays based on metal enhanced fluorescence (MEF-FIA) applicable as micro plate-, array-, multiplexing- or point of care-format.** Hawa G et al., Anal Biochem. 2018;549:39-44.

- **Wnt3a: Functions and Implications in Cancer.** Sha H et al., Chin J Cancer. 2015;34(12):554-62.

- **Elevated levels of Wnt3a and low levels of Dickkopf-1 in serum are associated with syndesmophyte formation in ankylosing spondylitis**. Klingberg E et al., Ann Rheum Dis. 2012;71:A64.

- **Expression of Wnt3a in hepatocellular carcinoma and its effects on cell cycle and metastasis.** Caijie L et al., Int J Oncol. 2017;51(4):1135-1145.

- **Wnt3a signaling within bone inhibits multiple myeloma bone disease and tumor growth**. Qiang YW et al., Blood. 2008;112(2):374-382.

- **The Importance of WNT Pathways for Bone Metabolism and Their Regulation by Implant Topography.** Galli C et al., Eur Cell Mater. 2012;24:46-59.

- **Active and water-soluble form of lipidated Wnt protein is maintained by a serum glycoprotein afamin/α-albumin.** Mihara E et al., eLife. 2016;5:e11621.

- **Wnt3a involved in the mechanical loading on improvement of bone remodeling and angiogenesis in a postmenopausal osteoporosis mouse model**. Li X et al. , FASEB J. 2019 Aug;33(8):8913-8924

- **Repair effect of Wnt3a protein on the contused adult rat spinal cord**.Yin ZS et al. Neurol Res. 2008 Jun;30(5):480-6.

13) NOTES

This page is left blank intentionally to be used for your personal notes regarding the assay.