FluoBolt™-NOGGIN

METAL ENHANCED FLUORESCENCE IMMUNOASSAY for FREE, BIOACTIVE NOGGIN

METAL ENHANCED FLUORESCENCE IMMUNOASSAY FOR
THE QUANTITATIVE DETERMINATION OF FREE, BIOACTIVE
NOGGIN IN HUMAN SERUM AND PLASMA

CAT. NO. FIA-1701-F,-C3,-C5,-A6 96 Well Formate

FOR RESEARCH USE ONLY

CE

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CONTENT

FIANOSTICS
Fluorescence Immunoassays

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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) NOGGIN

Noggin is a secreted homodimeric glycoprotein that is an antagonist of bone morphogenetic proteins (BMPs). Human Noggin cDNA encodes a 232 amino acid (aa) precursor protein; cleavage of a 19 aa signal peptide generates the 213 aa mature protein which contains an N-terminal acidic region, a central basic heparin-binding segment and a C-terminal cysteine-knot structure. Secreted Noggin probably remains close to the cell surface due to its binding of heparin containing proteoglycans. Noggin is very highly conserved among vertebrates.

Noggin predominantly binds BMP-4 and BMP-2 and antagonizes their bioactivities by preventing binding to both type I and type II receptors. During embryogenesis Noggin is a crucial factor for regulation of various developmental processes like formation of neural tubes, cardiomyocyte growth and patterning as well as skeletal development where Noggin prevents chondrocyte hyperplasia, thus allowing proper formation of joints. Mutations within the cysteine-knot region of human Noggin are linked to multiple types of skeletal dysplasias that result in apical joint fusions. Noggin is expressed in defined areas of the adult central nervous system and peripheral tissues such as lung, skeletal muscle and skin.
In adults NOGGIN may be associated with dissemination of tumor cells to bone, ankylosing spondylitis or pulmonary arterial hypertension (PAH). Its value as biomarker remains to be established yet.

3) CONTENT OF THE KIT

ID	KIT COMPONENT	QUANTITY
DM	Mouse monoclonal anti-human NOGGIN antibody, pre-coated MEF-microtiter plate, packed in an aluminium bag	1 x 96 well
WP	Wash buffer concentrate 20x, natural cap	1 x 25 ml
DAF, DA3, DA5, DAA	Rabbit polyclonal anti-human NOGGIN antibody, labeled with FITC or Cy3 or Cy5 or AlexaFluor680, black vial, black cap, ready to use	1 x 2.5 ml
DS	Standards 1-6, (0, 16, 31, 63, 125, 250 pmol/l), white caps, lyophilized	6 vials, 0.25 ml
DCA/B	Control A and B, yellow cap, lyophilized (for concentrations see label)	2 vials, 0.25 ml
DD	Sample diluent, natural cap, ready to use	1 x 10 ml

4) ADDITIONAL MATERIAL SUPPLIED WITH THE KIT

- 2 self-adhesive plastic films
- Protocol sheet
- Instruction manual for use
- Desiccant bag

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. For longer storage aliquot samples and store at -25°C or lower. Do not freeze-thaw samples more than 4 times.

Lipemic or haemolysed 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 DS (Standards) and DC (Controls). Leave at room temperature (18-26°C) for 20 min. Reconstituted DS and DC are stable at -25°C or lower until expiry date stated on the label. Reconstituted DS and DC can undergo 4 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 to run samples and standards in duplicates.

Take the plasmonic enhanced microtiter plate out of the aluminium 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 (DAA) because 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. 670/720nm). 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 detection antibody.

1) Add 25 µl of the selected fluorescence labeled detection antibody (DAF or DA3 or DA5 or DAA) 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 for 4 hours at 37°C or over night at room temperature (18-26°C) in the dark.

3a) If your reader allows bottom reading, read the plate without any further processing at the Ex/Em wavelength fitting to the delivered detection antibody (495/518 nm for DAF, 550/570 nm for DA3, 650/670 nm for DA5, 679/702 nm for DAA). Gain should be set to achieve at least 10000 fluorescence units (F.U.) between the signal of the 0 pM and the 250 pM NOGGIN standard. Samples with signals exceeding the signal of the highest standard must be re-run with an appropriate dilution using sample diluent (DD).

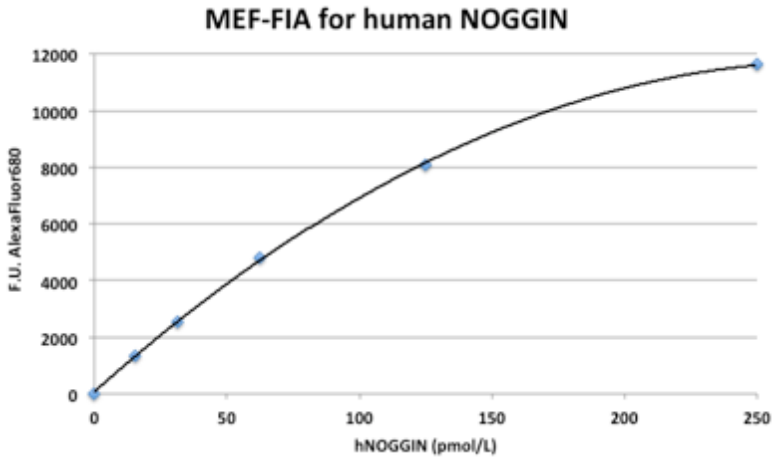
3b) If your reader has no bottom read option or if you want to store the plate for documentation purposes, discard or aspirate the content of the wells and wash 3x with diluted wash buffer. Use a minimum of 200 µl wash buffer per well. After the final wash, remove remaining fluid by strongly tapping 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 detection antibody (495/518 nm for DAF, 550/570 nm for DA3, 650/670 nm for DA5, 679/702 nm for DAA). Gain should be set to achieve at least 10000 fluorescence units (F.U.) between the signals of the 0 pM and the 250 pM NOGGIN standard. Samples with signals exceeding the signal of the highest standard must be re-run with appropriate dilution using sample diluent (DD).

4) Store the plate with desiccant at 4°C (2-8°C) in the aluminium 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 month, depending on signal intensity achieved.

8) CALCULATION OF RESULTS

Subtract the fluorescence intensity of the 0 pM 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. The assay was evaluated with 4PL algorithm. Different curve fitting methods need to be evaluated by the user.

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 250 pmol/l (6 standards and 2 controls in a human serum based matrix)
Conversion factor	1 pg/ml = 0.015 pmol/l (MW: 66 kD)
Sample volume	20 µl (undiluted sample) / well
Incubation time / temperature	4 h / 37°C or over night / RT
Sensitivity	LOD (0 pmol/l + 3 SD): 1 pmol/l; LLOQ: 5 pmol/l
Specificity	This assay detects free, bioactive NOGGIN, that is not complexed with BMP-2, -4 or -7
Cross-reactivity	NOGGIN is highly conserved across species. Human NOGGIN shares 99%, 99%, 98%, 97% and 89% aa sequence identity with mouse, rat, bovine, equine and chicken NOGGIN. Cross reactivity of this assay with other species than human has not been tested.

Precision:

Intra-assay (n=3)	**Sample 1**	**Sample 2**	**Sample 3**	**Sample 4**
Mean (pmol/l)	180	703	81	349
SD (pmol/l)	7,0	70,1	8,2	23,9
CV (%)	4%	10%	10%	7%
Inter-assay (n=3)	**Sample 1**	**Sample 2**	**Sample 3**	**Sample 4**
Mean (pmol/l)	155	81	36	32
SD (pmol/l)	3,5	4,9	2,5	1,4
CV (%)	2%	6%	7%	5%

Intra-assay: 4 samples of known concentrations were tested 3 times within 1 assay run

Inter-assay: 4 samples of known concentrations were tested in duplicates within 3 different assay runs

Spike/Recovery:

The recovery of NOGGIN was evaluated by adding 2 known concentrations of human recombinant NOGGIN to 4 different human serum samples. Mean recovery was 113% (see table below).

	Sample ID			
Spike (pM)	**#1**	**#2**	**#3**	**#4**
140	254	136	181	162
70	111	56	65	80
0	18	5	0	0
Target (pM)	158	145	140	140
	88	75	70	70
% Recovery	**161%**	**94%**	**129%**	**116%**
	126%	**75%**	**93%**	**114%**

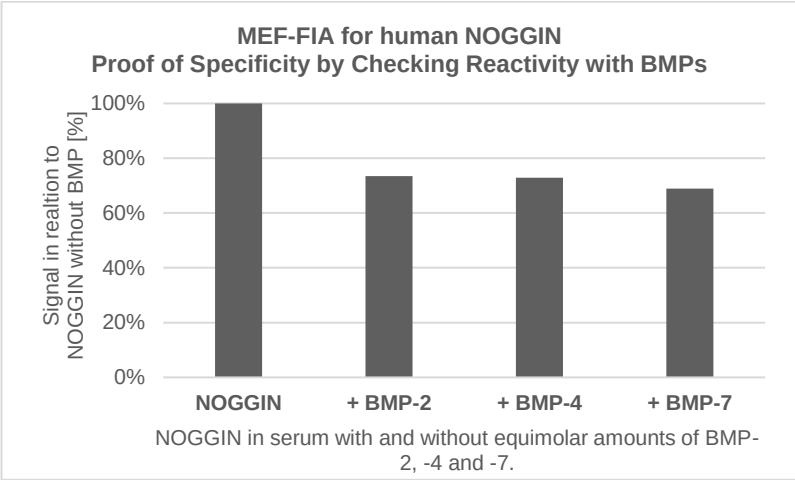
Linearity:

3 human serum samples were spiked with recombinant NOGGIN and diluted 1+1 and 1+2 with the sample diluent (DD) supplied with the kit. Mean linearity was 113% (see table below).

	Measured (pM)		
Dilution	**Sample #1**	**Sample #2**	**Sample #3**
1+0	64	155	498
1+1	26	84	485
1+2	18	46	91
	Expected (pM)		
1+1	32	78	249
1+2	21	52	166
	Linearity (%)		
1+1	123%	92%	51%
1+2	119%	112%	182%

Specificity:

Analyte Specificity:
This assay detects free, bioactive human NOGGIN, that is not bound to BMPs. Specificity was tested by titration of the serum based kit-calibrators with human recombinant BMP-2, -4 and -7.



As can be seen from the chart, addition of BMPs reduces the signal of the calibrator, which demonstrates that the assay only detects free, bioactive NOGGIN.

Species Specificity:
This assay has been exclusively tested with human serum and plasma samples. No data are available for other species. However human NOGGIN shares 99%, 99%, 98%, 97% and 89% aa sequence identity with mouse, rat, bovine, equine and chicken NOGGIN. So using this assay for those species 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

- **Metal-enhanced fluorescence: an emerging tool in biotechnology.** Aslan K, Gryczynski I, Malicka J, Matveeva E, Lakowicz JR, Geddes CD. Curr Opin Biotechnol, Feb 2005; 16(1):55-62.

- **Noggin attenuates the osteogenic activation of human valve interstitial cells in aortic valve sclerosis.** Poggio P, Sainger R, Branchetti E, Grau JB, Lai EK, Gorman RC, Sacks MS, Parolari A, Bavaria JE, Ferrari G. Cardiovasc Res, Jun 2013; 98:402-410.

- **The role of the BMP signaling antagonist noggin in the development of prostate cancer osteolytic bone metastasis.** Secondini C, Wetterwald A, Schwaninger R, Thalmann GN, Cecchini MG. PLoS One, Jan 2011; 6(1):e16078.

- **NOGGIN: a new therapeutic target for PH? Focus on "Noggin inhibits hypoxia-induced proliferation by targeting store-operated calcium entry and transient receptor potential cation channels".** Boucherat O, Bonnet S. Am J Physiol Cell Physiol, Jun 2015; 308(11).

- **Imbalance Between Bone Morphogenetic Protein 2 and Noggin Induces Abnormal Osteogenic Differentiation of Mesenchymal Stem Cells in Ankylosing Spondylitis**. Xie Z, Wang P, Li Y, Deng W, Zhang X, Su H, Li D, Wu Y, Shen H. Arthritis Rheumatol. Feb 2016; 68(2):430-40.

13) NOTES

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