CXCL 12 / SDF-1 Alpha ELISA

Catalog Number:
CXC31-K01
1 x 96 well ELISA kit
For Research Use Only
v. 1.1 (2.12.19)

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1. Introduction

The Eagle Biosciences CXCL 12 / SDF-1 Alpha ELISA Assay kit is an Enzyme-linked immunosorbent assay used for the quantitative and very sensitive determination of CXCL 12 / SDF-1 Alpha in serum and plasma. The CXCL 12 / SDF-1 Alpha ELISA Assay kit is for research use only and should not be used in diagnostic procedures.

SDF-1 Alpha (CXCL12) is a CXC chemokine [1]. The small basic protein (ca. 8 kDa) has no glycosylation site in its sequence [2]. The homology properties of SDF-1 Alpha are unique among the chemokines. It has a high degree of conservation within the mammalians, with 99 % identity between the human and the murine SDF-1 Alpha sequences. But it shows a weak homology to the other CXC chemokines and the SDF-1 gene is on chromosome 10, whereas the other chemokine genes are on chromosome 4 and 17 [3].

Except for SDF-1 Alpha there is another isoform of this chemokine, SDF-1 Beta. SDF-1 Alpha and SDF-1 Beta result from alternative splicing [1].SDF-1 Alpha binds to and activates the receptors CXCR4 and CXCR7 [1], whereupon SDF-1 Alpha is the only known ligand for CXCR4 [2]. SDF-1 Alpha binds also to fibronectin-1 [4], several glycosaminoglycans and heparin [5]. SDF-1 Alpha is constitutively expressed by bone marrow stromal cells [1] and many other tissues (for example heart, liver, brain, muscle, kidney) [3]. The function of SDF-1 Alpha is multifaceted. It plays a role in the prenatal development of the neural, hematopoietic and cardiovascular systems and is involved in the postnatal migration of CXCR4- and CXCR7-positive stem cells [1]. Furthermore, it has a chemo-attractant effect on CXCR4-positive lymphocytes [1] and plays a role in the growth and the metastasis of CXCR4- and CXCR7-positive tumors [6]. Except for the chemo-attractant effects SDF-1 Alpha has an inhibiting effect on HIV-1 *in vitro* [7].

2. Principle of the test

The biotinylated antibody of the CXCL 12 / SDF-1 Alpha ELISA Assay kit binds to the streptavidin coated microtiterplate. The SDF-1 α of the standards/samples binds to this antibody and is detected by the primary antibody. The biotinylated antibody, the primary antibody and the standards/samples are incubated on the microtiterplate altogether at the same time. The primary antibody, which is bound to SDF-1 Alpha, is then detected by a secondary peroxidase-conjugated antibody. The reaction with the substrate is stopped by addition of acid and the blue colour turns to yellow. The measured absorbance is directly proportional to the quantity of SDF-1 Alpha.

3. CXCL 12/SDF-1 Alpha ELISA Kit contents and storage

- Microtiter Plate: 96 wells (12 x 8 well strips in a foil pouch with desiccant), coated with streptavidin.
- Biotinylated Antibody: antibody to human SDF-1 Alpha, 6 µg, lyophilized, reconstitute in 60 µl Assay buffer, dilute 1:100.
- Monoclonal Antibody: antibody to human SDF-1, 360 µl (100 µg/ml)
- Standard, human SDF-1 alpha, 1 µg, lyophilized.
- Conjugate: Goat-anti mouse IgG-Peroxidase conjugate, 50 µl, dilute 1:2000
- Assay buffer: 40 ml, ready for use
- Washing buffer: 20 ml, 10-fold concentrated
- Substrate: TMB, 12.5 ml, ready for use
- Stop solution: 0,2 M sulfuric acid, 12.5 ml, ready for use (Caution: caustic)
- Instructions for use
- Store the CXCL 12/SDF-1 Alpha ELISA kit at 2-8°C.

4. Materials required, but not supplied

- Distilled or deionized water
- Disposable test tubes for sample preparation
- Pipettes
- Vortex mixer
- ELISA Reader

5. Precautions for Users

- The CXCL 12 / SDF-1 Alpha ELISA is for research use only
- The assay buffer contains bovine serum albumin. Therefore it should be treated as infectious materials and precautions should be taken as e.g. adequate clothing, wearing gloves etc. All waste should be disposed following the regulations for infective materials.
- The stop solution of the CXCL 12 / SDF-1 Alpha ELISA Assay kit contains acid that is harmful. Avoid contact with skin, eyes and mucous membranes.
- TMB Substrate is a suspected carcinogen. Avoid contact with skin, eyes and mucous membranes.
- The reagents of the CXCL 12 / SDF-1 Alpha ELISA Assay kit contain a preservative in very low concentration. Avoid contact with skin, eyes and mucous membranes.

6. Test procedure

6.1 Preparation of reagents and working solutions

6.1.1
Bring all reagents and the microtiter plate to room temperature. Remove the required quantity of strips from the microtiter plate. Strips not to be used immediately must be stored in the sealed bag with the desiccant at 4 °C.

6.1.2

Reconstitute the standard with 250 µl of the Assay Buffer. The reconstituted standard is stable for 5 days at 4 °C or for 1 month at -20 °C. Avoid repeated freeze-thaw cycles.

6.1.3
Reconstitute the biotinylated antibody with 60 µl Assay buffer (stock solution). Mix carefully and keep at room temperature for at least 30 minutes to **ensure complete dissolution.** The reconstituted antibody is stable for one month at 4 °C.

6.1.4
Preparation of the Antibody working solution: The antibody working solution should be prepared as follows

- Spin down the contents of the vials with the reconstituted Capture Antibody and the Detection Antibody solution by centrifugation and transfers contents into a vial with 6 ml Assay buffer. Use the same day.
- For a half plate: Mix 3 ml of assay buffer with 30 µl biotinylated antibody and 180 µl monoclonal antibody solution.

6.1.5
Conjugate: Dilute 1 + 2000 with Assay buffer, e.g. 6 µl plus 12 ml Assay buffer for 1 plate. Use the working solution the same day

6.1.6
Prepare the required amount of washing buffer by dilution of 1 volume of the concentrated washing buffer with 9 volumes of distilled or demineralized water. Use within one week.

6.2 Preparation of standards

- Standard 1 (40 ng/ml): Mix 10 µl Reconstituted Standard with 990 µl Assay buffer
- Standard 2 (20 ng/ml): Mix 500 µl Standard 1 with 500 µl Assay buffer
- Standard 3 (10 ng/ml): Mix 500 µl Standard 2 with 500 µl Assay buffer
- Standard 4 (5 ng/ml): Mix 500 µl Standard 3 with 500 µl Assay buffer
- Standard 5 (2.5 ng/ml): Mix 500 µl Standard 4 with 500 µl Assay buffer
- Standard 6 (1.25 ng/ml): Mix 500 µl Standard 5 with 500 µl Assay buffer
- If overnight incubation is used:
    - Standard 7 (0.625 ng/ml): Mix 500 µl Standard 1 with 500 µl Assay buffer

6.3 Assay Procedure

6.3.1

- Dispense 100 µl of assay buffer in Well A1 and A2 (Blank) of the microtiter plate (Blank).
- Dispense 50 µl of the assay buffer into the other wells of the microtiter plate.
- Dispense 50 µl of the standards and samples in duplicates per well.
- Dispense 50 µl of antibody working solution in each well
- Incubate for one hour or overnight at room temperature (22-25 °C).

Note: a constant temperature is important for a low coefficient of variation. Avoid draughts. Incubation is best done in a closed incubator with constant temperature.

6.3.2
Wash all wells three times with working wash solution:

- Automatic plate wash: Set plate washer to dispense 250 µl of washing buffer per well and a minimum of 20 seconds per washing step. Fill and aspirate for 3 cycles.
- Manual wash: Decant the contents of the wells by inverting sharply. Dispense 250 µl of diluted wash buffer to all wells. Decant and repeat twice. When washing is done manually, tap the inverted plate firmly on absorbent tissue to ensure complete removal of washing buffer before proceeding to the next step.

6.3.3
Dispense 100 µl conjugate working solution per well and incubate for one hour at room temperature.

6.3.4
Wash all wells three times as described in 6.3.2

6.3.5
Dispense 100 µl substrate solution per well. To minimize imprecision, this should be done by a multichannel pipette or in timed intervals. Incubate for 15 minutes at room temperature.

6.3.6
Stop reaction by adding 100 µl of stop solution to each well. To minimize imprecision, this should be done by a multichannel pipette or in timed intervals as in step 6.3.5. The blue colour will turn yellow upon addition of the stop solution.

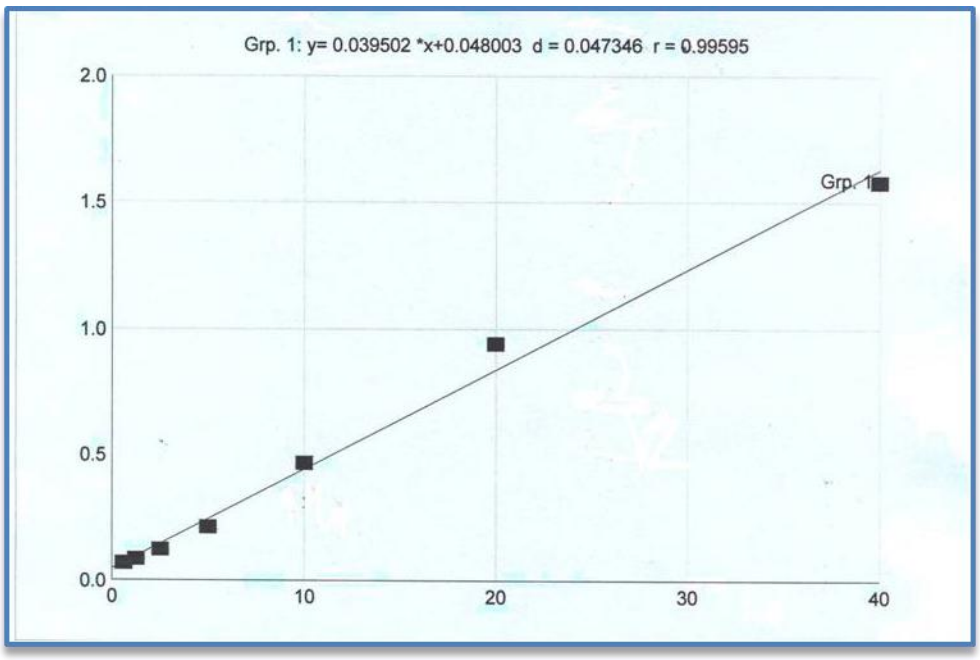
6.3.7
Measure the absorbance of each well at 450 nm (reference 650 nm).

7. Calculation of the results

The results are calculated by linear regression. The standard curve is plotted by drawing the regression curve with the absorbance on the y-axis and the concentration on x-axis. Calculate the results of the samples and multiply by the dilution factor.

8. Typical Test Results

Typical standard curve (not to be used for calculation of actual results):



9. Validation of the assay

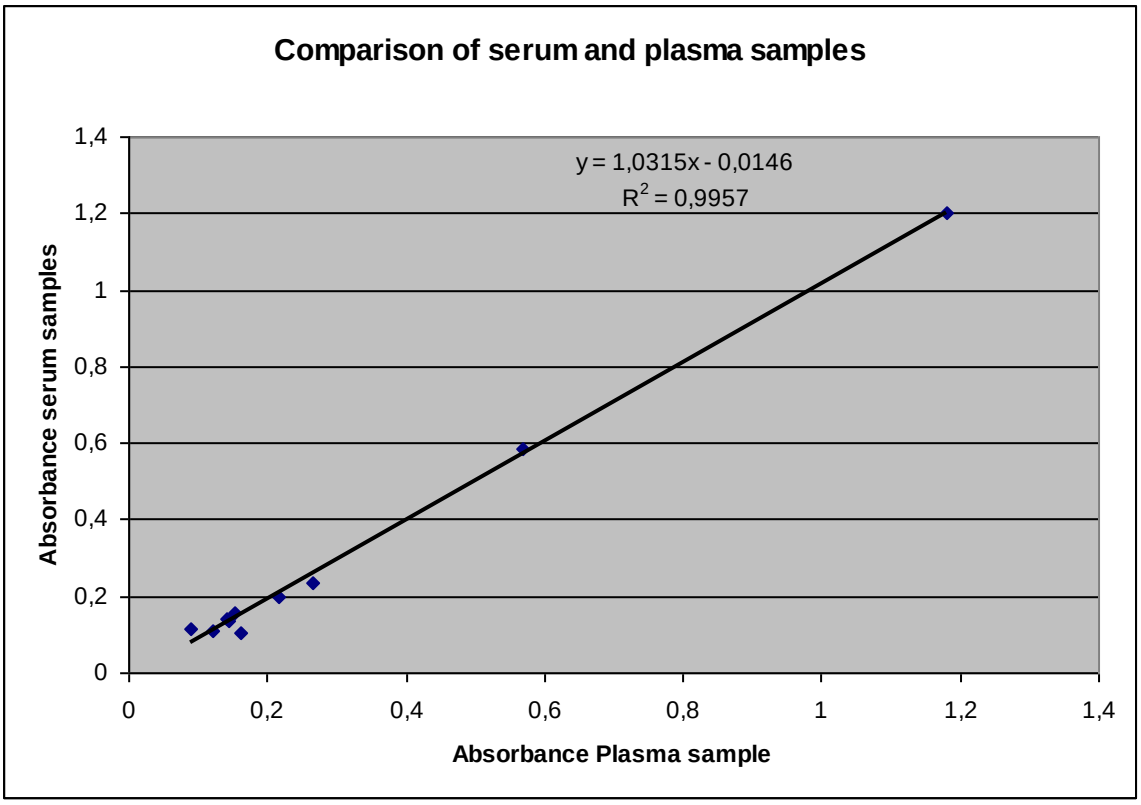
CXCL 12/SDF-1 Alpha ELISA Performance characteristics

Expected SDF-1 alpha levels in Normal and Disease State Sera:

	Mean sdf-1 alpha Concentration (ng/ml)	Range
Normal sera (n = 10)	2.86	0.31 – 15.1
Tumor sera (n = 13)	20.31	0.82 - 75.62
Diabetes Sera (n = 10)	11.63	1.17 – 39.23
Rheuma Sera (n = 10)	18.65	2.90 – 60.37

Serum-Plasma-Pairs:

10 serum plasma pairs (EDTA-plasma) have been tested with the CXCL 12 / SDF-1 Alpha ELISA. The correlation between the absorbance values of plasma and serum was 0.9957.



Linearity:
To assess the linearity of the CXCL 12 / SDF-1 Alpha ELISA, a sample containing an elevated concentration of SDF-1 alpha was diluted with assay buffer to produce samples with values within the range of the standard curve:

Sample dilution	Measured concentration, Recalculated, ng/ml
1:2	16.5
1:2,5	14.98
1:5	16.8
1:10	15,324
Mean Value/CV	15.878 / 6.01 %

Interfering Substances:
No interference has been observed in spiked samples with up to 1mg/ml bilirubin, 5 mg hemoglobin and 4 mg/ml triglycerides. Anyway, we do not recommend using highly lipemic or grossly hemolyzed samples.

Intra-assay Precision:
The intra-assay precision was determined by testing plasma samples 9 times on one plate. The CV was 0.80 % (sample 1) and 2.24 % (sample 2).

Inter–assay Precision:
The intra-assay precision was determined by testing plasma samples (4-fold determinations) on five days. The Inter-assay precision was 8.37 %.

Sensitivity:
The minimum detectable dose was determined by adding two standard deviations to the mean optical density value of the zero-standard and calculating the corresponding concentration. The minimum detectable dose for the CXCL 12 / SDF-1 Alpha ELISA was 0.31 ng/ml.

10. References

[1] Takahashi M (2010) "Role of the SDF-1/CXCR4 System in Myocardial Infarction." Circulation Journal 74 (3): 418-423
[2] Rollins BJ (1997) "Chemokines." Blood 90: 909–928
[3] Bleul CC et al (1996) "A Highly Efficacious Lymphocyte Chemoattractant, Stromal Cell-derived Factor 1 (SDF-1)." Journal of Experimental Medicine 184: 1101-1109
[4] Pelletier AJ et al. (2000) "Presentation of chemokine SDF-1a by fibronectin mediates directed migration of T cells." Blood 96: 2682-2690
[5] Mbemba E et al (2000) "Glycan and glucosaminoglycan binding properties of stromal cell-derived factor (SDF)-1 Alpha." Glycobiology 10 (1): 21-29
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[7] Maréchal V (1999) "Opposite Effects of SDF-1 on Human Immunodeficiency Virus Type 1 Replication." Journal of Virology 73 (5): 3608-3615.

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