GDF-15 (NAG-1, MIC-1) Cardiotoxicity ELISA kit

Catalog Number: **GDF15E**
Store at -20°C.
FOR RESEARCH USE ONLY

Introduction

This sandwich ELISA kit is for determination of GDF-15 (NAG-1, MIC-1) levels in biological samples and cell culture supernatants.
A typical standard curve of the ELISA (**detection limit =50 ng/ml**) is shown on page 6. Instructions are provided as to the proper isolation and purification of samples in the following pages.

The 96-well ELISA plate is coated with a monoclonal antibody specific for GDF-15 (NAG-1, MIC-1). Standards or samples are then applied to the wells to allow for binding of GDF-15 to the coated antibody. The wells are then washed to remove unbound substances and a second antibody (detection antibody), a polyclonal antibody which recognizes GDF-15 and which is conjugated to HRP (horseradish peroxidase) is added to the well. The wells are washed once more and a substrate solution for the peroxidase enzyme, TMB, is added to the wells. The TMB reacts with the HRP available in the well to form a blue-colored product. With the addition of sulfuric acid, the blue color is converted into a yellow-colored product, which can be read on a plate reader at 450 nm.

Storage and Stability

This kit will obtain optimal results if all of the components are stored at the proper temperature prior to use. Items should be stored at the designated temperatures upon receipt of this kit.

All components are stored below -20°C and should not be re-frozen and thawed more than necessary.

Precautions

- Please read all instructions carefully before beginning the assay.
- The reagents in this kit have been tested and formulated to perform optimally. This kit may not perform correctly if any of the reagents are replaced or any of the procedures are modified.
- This kit is intended for research use only and is not to be used as a diagnostic.

Materials Provided

Part Number	Item	Description	Quantity
1	GDF-15 ELISA Plate	Solid 96-well plate coated with anti-BPA antibody in each well	1
2	GDF-15 Standard (2 µL)	Stock standard at a concentration of 10 ug/mL	1
3	Detection Antibody (5 µL)	3000 X concentrated solution	1
4	Sample Dilution Buffer (25 mL)	20 X solution of Tris-buffered saline with preservatives	1
5	Streptavidin-HRP (3 mL)	5,000 X concentrated solution	1
6	Wash Buffer Solution (25 mL)	20 X solution of Tris-buffered saline with detergents and preservatives	1
7	TMB Substrate (22 mL)	A solution of TMB (tetra methyl benzadine)	1

Additional Required Materials **(Not Provided)**

- An 8-channel adjustable pipetter and an adjustable pipetter
- Storage bottles
- Costar® cluster tubes (1.2 mL) and microcentrifuge tubes
- Deionized water
- 2N Sulfuric acid

Procedural Notes

- Remove all of the reagents required, including the TMB, and allow them to equilibrate to room temperature before proceeding with the assay.
- It is necessary to thoroughly mix the concentrated buffer solutions. A stir bar is contained within each buffer solution.

Sample Preparations

There are different protocols for isolating BPA depending on the nature of the biological sample. Listed below are the different protocols. For optimal results follow the appropriate protocol based on the biological sample .

NAG-1 measurement in plasma or serum

1. Dilute the serum 4 to 20-fold with the Sample Dilution Buffer
2. Perform the ELISA for GDF-15 (according to the instructions of the manufacturer).

NAG-1 measurement in cells

1. Collect the cell media
2. Dilute 4-fold with sample dilution buffer
3. Perform the ELISA for GDF-15 (according to the instructions of the manufacturer).

Assay Preparations

The solid 96-well plate and TMB solution are provided ready to use. The preparations of other assay reagents are detailed below.

Wash Buffer:. Dilute the entire contents of the Wash Buffer Concentrate (25 mL) with 475 mL of deionized water to yield a final volume of 500 mL of 1 X Wash Buffer. This can then be refrigerated for the entire life of the kit.

Detection Antibody: Dilute the 5 uL of 3000X detection antibody in the vial with 15 mL of 1 X sample dilution buffer. One vial makes enough conjugate for one plate. The conjugate must be used the same day and should not be stored for later use.

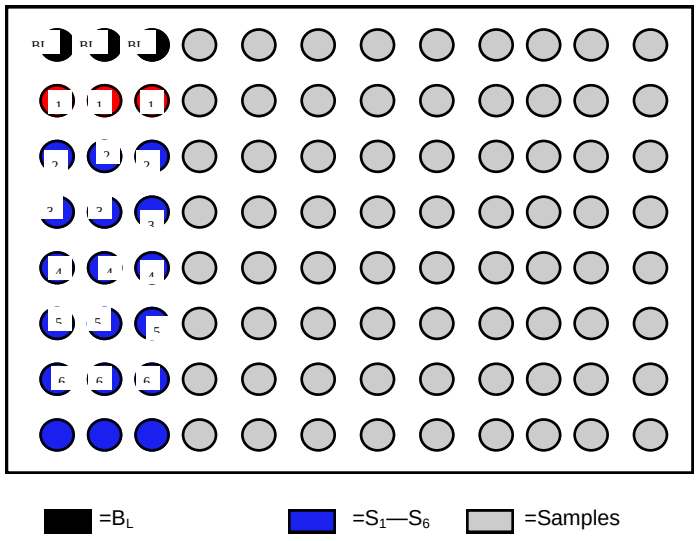
Standards: Label 6 microtubes as Standard 1 through Standard 5. Dilute the entire contents of Sample Dilution Stock buffer (25 mL) with 475 mL deionized water to yield a final volume of 500 mL of 1 X Sample Dilution Buffer. . Spin down the enclosed NAG-1 standard vial (2 µL, filled with inert gas) and add 1.998 mL of Sample Dilution Buffer to obtain 2 mL of solution. Label this Standard 6. Perform a serial dilution of the standards beginning with standard 6 as shown in the Standards Dilution Table on Page 4.

Streptavidin-HRP conjugate: Dilute the 3 uL of the 5,000X streptavidin-HRP conjugate supplied in the vial with 15 mL sample dilution buffer

Samples: Samples can be directly diluted into the 1 X Sample Dilution Buffer. The sample solution can be further diluted to a proper range of concentration for ELISA test.

Performing the Assay

Plate Setup: Each plate must contain a minimum of three blank wells (B_L), and a seven point standard curve (S_1-S_6). Each sample should be assayed in triplicate. A suggested plate format is shown below:



Standard Dilutions Table

Standards	Final Concentration (pg/mL)	Add Sample Dilution Buffer (mL)	Serial Dilutions Procedure
No. 6	10,000	1.998	2 µL of stock solution.
No. 5	5,000	0.5	Add 0.5 mL of No. 6
No. 4	1000	0.8	Add 0.2 mL of No. 5
No. 3	500	0.5	Add 0.5 mL of No. 4
No. 2	100	0.8	Add 0.2 mL of No. 3
No. 1	50	0.5	Add 0.5 mL of No. 2

Assay Procedure

Step 1: Load 100 microliters of Sample Dilution Buffer into the blank (B_L) wells.

Step 2: Load 100 microliters of each of the standards into the appropriate wells.

Step 3: Load 100 microliters of each of the samples into the appropriate wells.

Step 4 Incubate the plate at room temperature for two hours.

Step 5: Wash the plate three times with 400 microliters of the diluted Wash Buffer per well.

Step 6: After the last of the three wash cycles pat the plate dry onto some paper toweling.

Step 7: Add 100 microliters of the detection antibody to all sample and standard wells EXCEPT FOR the B_L wells).

Step 8: Incubate the plate at room temperature for 2 hours

Step 9: Wash the plate three times with 400 microliters of the diluted Wash Buffer per well.

Step 10: Add 100 microliters of the streptavidin-HRP to all wells EXCEPT FOR the B_L wells

Step 11: Incubate the plate at room temperature for 1 hour

Step 12: Wash the plate three times with 400 microliters of the diluted Wash Buffer per well.

Step 13: Add 200 microliters of the TMB to ALL WELLS

Step 14: Incubate for 30 minutes at room temperature

Step 15: Add 50 microliters of the 2N Sulfuric Acid to all of the wells

Step 16: Read the plate at 450 nm.

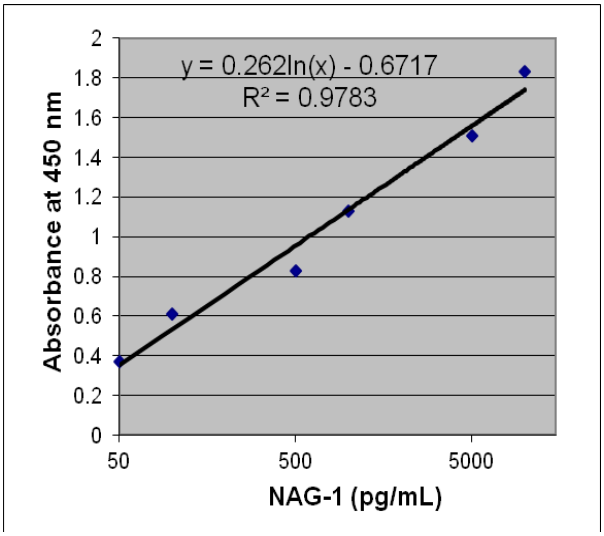
Calculating the Results

Most plate readers provide data reduction software that can be used to plot the standard curve and determine the sample concentrations. If your plate reader does not have this option, then a data reduction program can be used (4 parameter of log-log curve fit).

If you do not have these options, the results can be obtained manually as follows:

1. Average the absorbance readings from the blanks and subtract that value from each well of the plate to obtain the corrected readings. (Note: Some plate readers do this automatically. Consult the user manual of your plate reader.)
2. Average the corrected absorbance readings for each of the standards and blank wells.
3. Create a standard curve by plotting the mean net absorbance for each standard (y-axis) versus the standard concentrations (x-axis; log scale)
4. Use the formula generated by regression analysis to calculate the concentrations of the unknown samples.
5. Multiply the concentrations obtained for each of the samples by their corresponding dilution factor.

Typical Results



The data shown here is an example of typical results obtained using the Detroit R & D GDF-15 ELISA kit. These results are only a guideline, and should not be used to determine values from your samples. The user must run their own standard curve every time.

B_L wells = 0.047

Standard	Concentration	O.D.
No. 1	50 pg/mL	.416
No. 3	100 pg/mL	.658
No. 4	500 pg/mL	.876
No. 5	1000 pg/mL	1.17
No. 6	5,000 pg/mL	1.55
No. 7	10,000 pg/mL	1.88

Troubleshooting

No color present in standard wells.

- The streptavidin-HRP conjugate was not added. Redo the assay and add the conjugate at the proper step.
- The streptavidin HRP conjugate was not incubated for the proper time. Redo the assay and incubate for the proper time.

No color in any wells

- The TMB substrate was not added. Add substrate.
- The TMB substrate was not incubated for the proper time. Continue incubation until desired color is reached.

The color is faint.

- One or all of the incubation times were cut short. Redo the assay with the proper incubation times.
- The TMB substrate was not warmed up to room temperature. Redo the assay making sure all reagents are at room temperature.
- The lab is too cold. Be sure the lab temperature is between 21-27°C and redo the assay.

The background color is very high.

- The TMB substrate has been contaminated. Redo the assay with a fresh bottle of substrate.

Scattered O.D. obtained from the sample.

- Redo assay using an 8-channel pipetman making sure that 8 channels are equal volume while loading.

References

1. Welsh JB et. al. Large scale delineation of secreted protein biomarkers overexpressed in cancer tissue and serum. PNAS 100: 3410-3415 (2003)
2. Eling, TE et. al. NSAID activated gene (NAG-1), a modulator of tumorigenesis. J. Biochem. Mol. Biol. 39: 649-655 (2006)
3. Kim, H. 2011 US Patents: "Form specific antibodies for NAG-1 (MIC-1, GDF-15), H6D and other TGF-beta subfamily and heart disease and cancer diagnoses", pending.

Warranty

Detroit R&D, Inc., makes no warranty of any kind expressed, or implied, including, but not limited to the warranties of fitness for a particular purpose and merchantability.

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