Northwest
Life Science Specialties, LLC

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NWLSS™ Dityrosine ELISA

Product NWK-DIY01

For Research Use Only

This ELISA kit is designed for quantitative measurement of dityrosine (DiY) from any species in urine and other samples where it may be present.

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Introduction:

Oxidative stress is known to play an important role in the development of various diseases and aging processes. Under conditions of oxidative stress, certain amino acids are more or less susceptible to alterations by reactive oxygen species (ROS) and reactive nitrogen species (RNS). Among them, Cysteine, Methionine and Tyrosine have been widely investigated. Unlike, byproducts of Cysteine and Methionine oxidation, there are no biological mechanisms to repair oxidatively modified tyrosine so these byproducts are of particular interest in many diseases and aging. Nitrotyrosine, dityrosine, and dibromotyrosine have all been identified as potential protein related biomarkers of oxidative stress. Dityrosine, the subject of this new assay forms as a result of crosslinking of modified proteins. It has recently been shown to form preferentially over nitrotyrosine in the presence of low levels of peroxynitrite (ONOO-) and as such may prove to be another surrogate biomarker for ONOO– mediated damage. Dityrosine has been and continues to be investigated for its role in many biological processes including aging and age related diseases such as atherosclerosis, cataract formation and neurodegenerative diseases such as Parkinson's and Alzheimer's.

Intended Use:

The NWLSS™ Dityrosine ELISA is intended for quantitative detection of the Dityrosine protein dimer biological samples including urine, plasma, serum, tissue and other biological samples.

Test Principle:

The NWLSS™ Dityrosine ELISA assay uses a competitive format wherein a murine monoclonal antibody to Dityrosine (*Primary Antibody*) and sample or standard are added to a microtiter plate which has been precoated with Dityrosine. Sample or calibrator Dityrosine competes with plate-bound Dityrosine for binding with the Primary Antibody. Accordingly, higher concentrations of sample or calibrator leads to reduced binding of the antibody to the Dityrosine coated plate. A subsequent wash step removes any free Dityrosine/antibody adduct leaving stationary plate bound Dityrosine complexed to antibody for later detection. Anti-murine Secondary Antibody conjugated to horse radish peroxidase (*HRP-Conjugate*) is then added to the plate. HRP-conjugate binds to remaining murine anti-Dityrosine and unbound HRP-conjugate is removed in another wash step. Addition of 3,3',5,5'tetramethylbenzidine (*TMB Substrate*) results in blue color development proportional to the amount of anti Dityrosine antibody bound to the plate and inversely proportional to the concentration Dityrosine in original samples or calibrators applied to the plate. The reaction is terminated by addition of sulphuric acid (*Stop Solution*) producing yellow color with measurable absorbance at 450 nm.

General Specifications:

Format::	1 X 96 wells	
Number of tests:	Triplicate =	24
	Duplicate =	40
Specificity:	Di-Tyrosine	
Sensitivity:	LLD = 50 ng/mL	

Kit Contents:

Microtiter Plate:	Precoated with DiY	12 X 8 wells
Di-Y Standard:	6 levels Purified DiY	6 Vials (0.5mL ea)
Primary Antibody:	Mu-Anti-DiY Monoclonal	1 vial (7 mL)
Secondary Antibody:	Anti-murine-HRP conjugate	1 vial (Lyoph.)
Secondary Antibody Buffer:	Phosphate Buffered Saline	1 vial (12 mL)
TMB Substrate:	3,3',5,5'tetramethylbenzidine	1 vial (12 mL)
Wash Buffer (5x):	Concentrated PBS	3 vials (25 mL)
Stop Solution:	1.9% Sulfuric Acid	1 vial (12 mL)
Plate Seal		2 Sheets

Required Materials Not Provided:

Adjustable pipettes capable of transferring 50 µL to 200 µL volumes.
A multi-channel or repeater pipette (recommended).
Saline (0.9% NaCl in distilled water)
Reagent Trays (if using multi-channel).
4-10°C Incubator
Distilled water

Required Instrumentation:

Microplate reader with 450nm capability.

Warnings, Limitations, Precautions:

Incubation Temperature

Measured values can be affected by variations in incubation temperatures. Care should be exercised to assure uniform temperature during incubations, particularly during the Primary Antibody reaction period. It is recommended that incubation be performed in a humid environment (i.e. in an incubator with water present.

Possible edge effects

To minimize edge effects, ensure that plate is sealed properly and that incubation times are as uniform as possible. To maintain the most uniform temperature within the wells, it is recommended that any unused wells on a single strip be filled with an equal volume of water prior to incubation.

Blank wells:

Do not add Primary Antibody to Blank wells.

Storage Instructions:

Upon receipt, store the reagents at 2-8ºC. This kit is stable if unopened for 12 months from the date of manufacture. Do not use components beyond the expiration date printed on the kit box label.

Unused wells and opened reagents must be stored at 2-8ºC and should be used within two weeks after first opening.

Assay Preparation

Plate Reader Setup

Wavelength:	450 nm
Mode:	Endpoint
Shaker:	On
Curve Fit:	4-Parameter Logistic

Reagent Preparation:

Bring all reagents, plate wells to be used samples and calibrators to room temperature (20-25°C) before use.

Wash Buffer:

Prepare the necessary volume of **Working Wash Buffer** by mixing Mix 1 part 5X Wash Buffer with 4 parts distilled water (eg 1 vial 5X Wash Buffer to 100 mL distilled water.

Note: Working Was Buffer is stable for one week at 4 degree C.

Secondary Antibody: (Blue Cap and Label)

Just prior to use add 1 vial of **Secondary Antibody Buffer** to **Secondary Antibody**, mix gently and let stand for 5 minutes at room temperature. Label as **Working Secondary Antibody**.

Note: **Working Secondary Antibody** is stable for 1 week at 4°C.

Sample Handling/Preparation:

The multi-disciplinary interest in measuring Di-Tyrosine will result in a myriad of sample types and experimental conditions and is beyond the scope of this product insert to describe sample processing in detail for each case. However, general guidelines are provided below for representative sample types.

Urine:

Mix with 3 volumes of saline before assay. Insoluble materials should be removed by centrifugation. Please note that samples must be diluted with saline (0.9% NaCl in distilled water).

Note: Washing buffer and PBS can't be used for sample dilution.

Assay Protocol:

Standard Procedure for Microplate Assay

Day 1:

1. Bring all reagents to room temperature.

2. Remove appropriate number of wells for assay from foil pouch. Remove seal from wells before use.

Note: Wells are glycerol coated as a preservative.

Note: Microtiter plate wells are stable for 1 week after opening the bag.

3. Construct an assay template to ensure proper sample addition.

4. Wash plate 3 times as follows:
Add 250µL **Working Wash Buffer** per well...Let stand 3 minutes at room temp.
Empty wash solution into sink by inversion then blot plate against clean paper towel to remove any remaining washing buffer.

4. Add 50µL of **Standard** or **Diluted Sample t**o each well to be assayed.

5. Add 100µL **Distilled Water** to blank wells.

6. Add 50µL **Primary Antibody** to each well to be assayed except blank wells.

7. Shake lightly side to side to ensure proper mixing.

8. Cover plate with adhesive strip, then incubate at 4-10°C overnight.

9. Return all reagents to 4°C refrigerator.

Day 2:

10. Bring all reagents to room temperature.

11. Empty contents of wells into sink and blot on paper towel to remove as much fluid as possible.

12. Wash plate 3 times as follows:
Add 250µL **Working Wash Buffer** per well
Empty wash solution into sink by inversion then blot plate against clean paper towel to remove any remaining washing buffer.

13. Add 100µL of **Working Secondary Antibody** per well.

14. Shake lightly side to side to ensure to mixing.

15. Cover the plate with an adhesive strip then incubate at 20-24°C for 1 hour.

16. Empty contents of wells into sink and blot on paper towel.

17. Wash the plate 3 times as in Step 12.

18. Add 100µL of **TMB Substrate** per well.

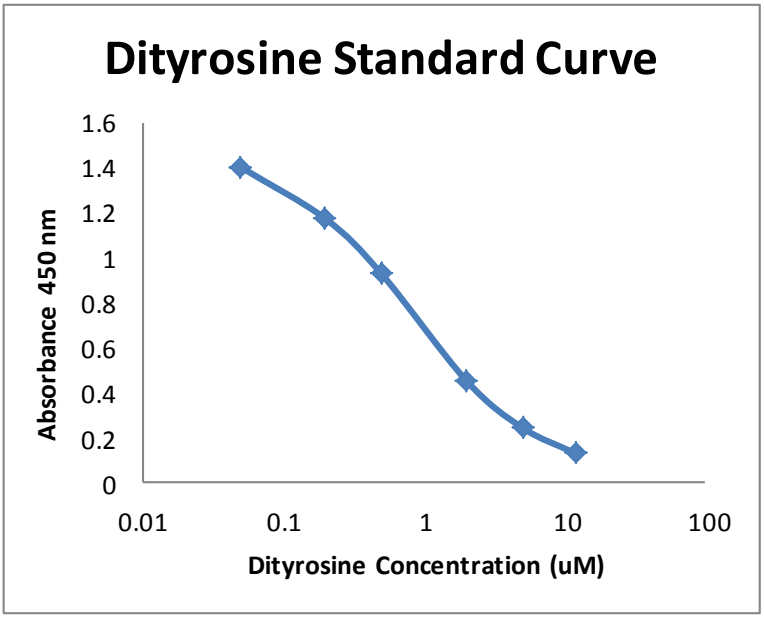
19. Shake lightly side to side to ensure proper mixing.

20. Incubate for 15 minutes, at room temperature in the dark.

21. Add 100µL of **Stop Solution** per well. Shake gently.

22. Measure the absorbance at 450nm.

Data Analysis:
Create a standard curve by plotting Absorbance vs. Concentration (log scale) for each standard level assayed. If available, set the plate reader to utilize 4-Parameter curve fit. An example standard curve is shown below.



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Statement of Limited Warranty:

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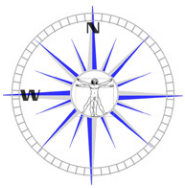
Notes:

Notes:



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