

**Northwest**  
**Life Science Specialties, LLC**

**Premier Products for Superior Life Science Research**

***NWLSS™***  
***Rat Nrf2 (NFE2L2)***  
***ELISA***

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**Product NWK-NRF2R1**

***For Research Use Only***

**Simple assay kit for quantitative measurement of Rat Nrf2 (NFE2L2)**

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**Introduction:**

Bridging the gap between normal redox homeostasis and oxidative stress lies a transcription factor protein known as nuclear factor, erythroid 2 like protein 2 (NFE2L2) commonly referred to as Nrf2. Nrf2 is a basic leucine zipper (bZIP) protein functioning to regulate the expression of many antioxidant proteins. Nrf2 can be found in the cytosol where it is bound to the Kelch-like ECH-associated protein 1 (KEAP1) regulatory molecule. In this sequestered condition Nrf2 cannot cross into the nucleus. However, under conditions of oxidative stress, KEAP1 can dissociate from Nrf2 allowing Nrf2 to translocate into the nucleus. Inside the nucleus Nrf2 binds to small Maf (sMAF) proteins forming an Nrf2/sMAF heterodimer capable of binding to the Antioxidant Response Element (ARE) on various stress related gene targets. In recent years Nrf2/KEAP1 regulation of the Nrf2/sMAF/ARE transcription pathway has been investigated extensively to assess its possible role in aging and disease. Many studies are showing a positive correlation between Nrf2 nuclear translocation and amelioration of disease resulting in increased interest in Nrf2 promoters and KEAP1 agonists as possible therapeutic agents. Other studies show paradoxical outcomes such as when considering Nrf2 in relation to cancer. In that case, it appears that some tumor cells are capable of exploiting the Nrf2 system to upregulate antioxidant expression to confer chemo-protective properties making the tumor more virulent and resistant to therapy. In either role Nrf2 has emerged as a very important target for researchers attempting to better understand the positive and negative effects of redox related stress response.

**Intended Use:**

The NWLSS™ Rat Nrf2 (NFE2L2) ELISA is intended for quantitative detection of Rat Nuclear Factor Erythroid 2 Like Protein 2, also known as Nrf2.

**Test Principle:**

The NWLSS™ Rat Nrf2 (NFE2L2) ELISA is a sandwich format Enzyme-Linked Immunosorbent Assay (ELISA). The assay features a microtiter plate pre-coated with a monoclonal antibody specific to rat Nrf2. Briefly, Nrf2 in samples and standards is captured by the plate bound antibody and remaining liquid is removed. Next, a biotinylated antibody with Nrf2 specificity is added and allowed to bind to Nrf2 captured in the previous step. After incubation, non-bound components are removed by washing. Avidin-HRP conjugate is then added and allowed to incubate. After a subsequent thorough wash TMB-substrate solution is added to each well which produces blue coloration wherever Nrf2 is present. Finally, a sulfuric acid stop solution is added and the resulting yellow colored product is measured at 450nm. The amount of Nrf2 in the sample can be easily determined by direct comparison of unknown sample absorbance with known standard curve absorbance values generated in the assay.

**General Specifications:**

Format::	1 X 96 wells
Number of tests:	Triplicate = 24 Duplicate = 40
Specificity:	Rat Nrf2 (NFE2L2)
Sensitivity:	LLD = 60 pg/mL in sample applied to plate
Standard Range:	0.156 ng/mL - 10 ng/mL

**Kit Contents:**

Foil Pouch	Microtiter plate precoated with anti-Rat Nrf2	12 X 8 wells
2 Vials	NFE2L2 (Nrf2) Standard (Purified Rat Nrf2)	(Lyophilized)
1 Bottle	Standard Diluent	(20 mL)
1 Vial	100X Secondary Antibody	(120 µL)
1 Bottle	Secondary Antibody Diluent	(12 mL)
1 Vial	100X Avidin-HRP Conjugate	(120 µL)
1 Bottle	Avidin-HRP Diluent	(12 mL)
1 Bottle	TMB Substrate (3,3',5,5'-tetramethylbenzidine )	(9 mL)
1 Bottle	Stop Solution: (1 N Sulfuric Acid, H <sub>2</sub> SO <sub>4</sub> )	(6 mL)
1 Bottle	30X Wash Buffer	(20 mL)
4	Adhesive Plate Covers	(Sheets)

**Required Materials Not Provided:**

Adjustable pipettes capable of transferring 50 µL to 200 µL volumes.  
A multi-channel or repeater pipette (recommended).  
Eppendorf tubes for sample & standard dilutions.  
Reagent Trays (if using multi-channel).  
37 °C Incubator  
Distilled water

**Required Instrumentation:**

Microplate reader with 450nm capability.

**Warnings, Limitations, Precautions:**

*Stability Limitation:*

The diluted Standards, Secondary Antibody and Detection Reagent should be used only once.

*Standards:*

Do not attempt to serially dilute the standards directly in the plate wells. Doing so will produce erroneous results. For best results, prepare the standards in separate tubes just prior to performing the assay.

*Incubation Temperature*

Measured values can be affected by variations in incubation temperatures. Exercise care to assure uniform temperature during each incubation. We recommend that incubation be performed in a humid environment such as in an incubator with water present.

*TMB Substrate:*

TMB Substrate is light sensitive: Keep it in the dark. Do not open until just before use.

*Wash Solution:*

If crystals have formed in the concentrated 30X Wash Solution, simply warm to room temperature and mix gently until the crystals are completely dissolved.

*Blank wells:*

Do not add Tracer Antibody to Blank wells.

*Safety:*

Individual components may be harmful if swallowed, inhaled or absorbed through the skin. Contact should be minimized through the use of gloves and standard good laboratory practices. If contact with skin or eyes occurs, rinse the site immediately with water and consult a physician.

**Storage Instructions:**

Upon receipt, the Standard, Tracer Antibody, Detection Reagent and the 96-well strip plate should be stored at -20 °C . The remaining components should be stored at 2-8 °C.

This kit is stable if unopened for 6 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 according to their recommended storage temperature and should be used within 30 days after first opening.

**Assay Preparation***Plate Reader Setup*

Wavelength:	450 nm
Mode:	Endpoint
Shaker:	On
Curve Fit:	2nd Order Polynomial

**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 wash buffer by mixing 1 part **30X Wash Buffer** with 29 parts distilled water. Label as **Working Wash Buffer**.

*Secondary Antibody:*

Briefly centrifuge the **100X Secondary Antibody** vial to ensure contents are fully recovered then dilute 1:100 with the **Secondary Antibody Diluent** to create the necessary volume. (e.g. 20 µL stock to 1.98 mL buffer for each 16 wells to be assayed). Label as **Working Secondary Antibody**.

*Avidin-HRP Conjugate:*

Briefly centrifuge the **100X Avidin-HRP Conjugate** vial to ensure contents are fully recovered then dilute 1:100 with the **Avidin-HRP Diluent** to create the necessary volume. (e.g. 20 µL stock to 1.98 mL diluent for each 16 wells to be assayed). Label as **Working Avidin-HRP**.

*TMB Substrate:*

**TMB Substrate** is provided ready for use. To avoid contamination, we recommend transferring the necessary volume to a separate container just prior to use. Do not dump the excess back into original container.

**Sample Handling/Preparation:**

The primary usefulness of this assay is expected to be in measuring Nrf2 levels in tissue homogenates and cell lysates.

**Tissue:**

The quantity of tissue required will vary by investigational model system and this information is recommended only as a general guide. Remove excess blood by rinsing tissue in ice-cold PBS (0.02mol/L, pH 7.0-7.2). Homogenize tissue in ice cold PBS using a glass homogenizer. It might also be necessary to sonicate the resulting homogenate to ensure cell membranes are suitably disrupted to allow for detection of Nrf2. Centrifuge the homogenates and harvest the clarified supernatant to be assayed. We have not fully verified the stability of Nrf2 after processing in this manner and recommend testing freshly harvested and processed samples whenever possible. If unable to test immediately, we recommend freezing at -80 °C

**Cells**

We recommend that cells be disrupted in a similar fashion as tissue, using a glass homogenizer in conjunction with sonication if necessary. Multiple freeze thaw cycles may also be used to disrupt the cells. We do not recommend using commercially available lysis buffers.

**Standard Curve Preparation:**

Reconstitute the Standard to 20 ng/mL by adding 1mL of **Standard Diluent** into the standard protein vial containing lyophilized rat Nrf2. Swirl or mix gently, and allow to sit for 5 minutes to ensure complete reconstitution. Use or freeze within 1 hour of reconstitution.

1. Label tubes 1-8 as:

10, 5, 2.5, 1.25, 0.625, 0.312, 0.156 and zero (0) ng/mL.

2. Add 500 µL **Standard Diluent** to each of tubes 1-8.

3. Add 500 µL Reconstituted stock 20 ng/mL Standard to tube 1 and mix well. Tube 1 is now 10 ng/mL

**Note: Unused reconstituted standard can be frozen at -70 °C and thawed one time only without significant loss of immunoreactivity.**

4. Make a serial dilution by transferring 500 µL of 10 ng/mL Standard (tube 1) into tube 2 mixing thoroughly then 500 µL of resulting 5 ng/mL to tube 3 and so on through to tube 7 to create all Standards down to 0.156 ng/mL.

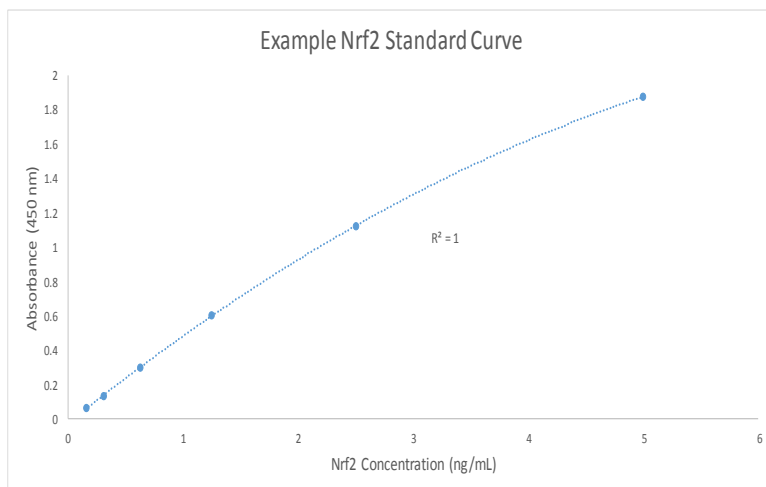
**Assay Protocol:***Standard Procedure for Microplate Assay*

1. Bring all reagents to room temperature.
2. Remove appropriate number of wells for assay from foil pouch.
3. Construct an assay template to ensure proper sample addition.
4. Add 100µL of **Sample** or **Standard** to each well to be assayed and 100 µL PBS to Blank wells.
5. Cover with Plate Seal then incubate 2 Hours at 37 °C.
6. Empty contents of wells into sink but do not wash the plate.
7. Add 100µL **Working Secondary Antibody** to each well to be assayed except blank wells.
8. Cover with Plate Seal then incubate for 1 Hour at 37 °C.
9. Empty contents from each well into sink and blot on paper towel to remove as much fluid as possible.
10. Wash plate 3 times as follows:  
Add 350µL **Working Wash Buffer** per well  
Empty wash solution into sink then blot plate against clean paper towel to remove any remaining wash buffer.
11. Add 100µL of **Working Avidin-HRP** per well.
12. Cover with Plate Seal then incubate at 37 °C for 30 minutes.
13. Empty contents of wells into sink and blot on paper towel.
14. Wash the plate 5 times as in Step 10.
15. Add 90µL of **TMB Substrate** per well.
16. Cover with Plate Seal then incubate at 37 °C for 15 - 25 minutes. Blue color development should be monitored and the reaction stopped before the highest standard becomes too dark (typically, an Abs 650 of around 1.2 will produce good results in the 450 nm range after stopping).
17. Add 50µL of **Stop Solution** per well.
18. Measure the absorbance at 450nm.



**Data Analysis:**

Create a standard curve by plotting Absorbance vs. Concentration ) for each standard level assayed. If available, set the plate reader to utilize quadratic (2nd order polynomial). An example standard curve is shown below.



**Note:** Inclusion of the high standard is optional. When samples are found to be in the lower portion of the curve, omission of the high standard can sometimes provide a more accurate curve fit and thus better results.

**References:**

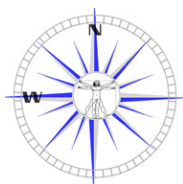
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**Statement of Limited Warranty:**

Northwest Life Science Specialties, LLC (NWLSS) makes no guarantee of any kind, expressed or implied, that extends beyond the description of the material in this kit, except that they will meet our specifications at the time of delivery. Customer's remedy and NWLSS' sole liability is limited to, at NWLSS' option, refund of the purchase price, or the replacement of material not meeting our specification. By acceptance of our product, customer assumes all liability and will indemnify and hold NWLSS harmless for the consequence of this product's use or misuse by the customer, its employees, or others. Refund or replacement is conditioned of customer notifying NWLSS within twenty-one (21) days of the receipt of product. Failure to give notice within 21 days shall constitute a waiver by the customer of all claims hereunder with respect to said product.

**Notes:**

**Notes:**



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