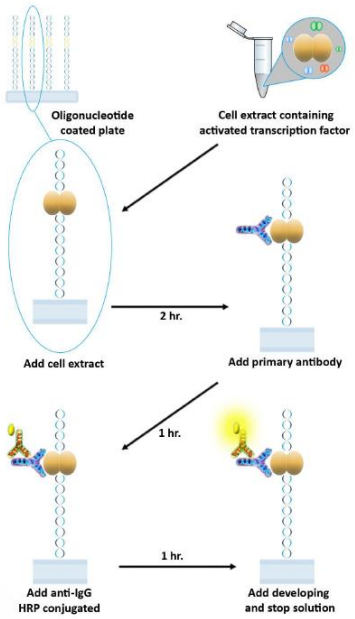
NRF2 (Human) Transcription Factor Activity Assay Kit

07/17

(Catalog # E4337-100, 100 assays, Store at -20°C)

I. Introduction:

Cellular oxidative and electrophilic stress caused by drugs and other xenobiotics, inflammation, and ionizing radiation are associated with an accumulation of reactive oxygen species and electrophilic insults, which contribute to the pathogenesis of various diseases such as cancer, neurodegenerative disease, and atherosclerosis. In order to protect cells from reactive oxygen species and electrophilic insults, the endogenous cellular antioxidant defense system initiates a response to cellular oxidative and electrophilic stress. NRF2 (nuclear factor (erythroid-derived 2)-like 2; NFE2L2) is a key transcriptional factor regulating hundreds of antioxidant and Phase II detoxification genes. Under normal conditions, NRF2 is sequestered in the cytoplasm through binding with Keap1, an actin-binding protein and finally is degraded through the Keap1-dependent ubiquitination. In response to a stimulus, degradation of Keap1 is markedly increased. This leads to the disruption of the Keap1-NRF2 complex and nuclear translocation of NRF2. NRF2 then dimerizes with small Maf proteins and binds to the ARE (antioxidative response element) in promoters of downstream genes to initiate expression. Accurate monitoring of the level of activated NRF2 in cells, tissues or animal models is required for investigating signal transduction pathways and other research applications such as drug development. Simple, speedy and high-throughput methods are required for this purpose. The BioVision NRF2 Transcription Factor-Activity Assay kit is a non-radioactive transcription factor assay with an ELISA format. It offers an easy, speedy, sensitive and high-throughput method to detect the activation of transcription factors.



Application:

This ELISA kit is used for in vitro quantitative determination of active NRF2

II. Sample Type:

Nuclear extraction, whole lysates

III. Kit Contents:

Components	E4337-100	Part No.	Storage after Preparation
Microplate	12x8 wells	E4337-100-1	4°C for one month
DNA Binding Buffer (5X)	4 ml	E4337-100-2	4°C
Positive Control	20 µl	E4337-100-3	-20°C /-80°C
Specific Competitor DNA Probe	1 vial	E4337-100-4	4°C
Non-specific Competitor DNA Probe	1 vial	E4337-100-5	4°C
Assay Reagent	200 µl	E4337-100-6	4°C
DTT (300 mM)	200 µl	E4337-100-7	4°C
Wash Buffer Concentrate (20X)	25 ml	E4337-100-8	4°C
NRF2 Primary Antibody	1 vial	E4337-100-9	4°C for 5 days
HRP-conjugated Secondary Antibody	1 vial	E4337-100-10	4°C for 5 days
Antibody Diluent Buffer	25 m	E4337-100-11	4°C
TMB One-Step Substrate Reagent	12 ml	E4337-100-12	4°C
Stop Solution	8 ml	E4337-100-13	4°C

IV. User Supplied Reagents and Equipment:

- Protease and Phosphatase inhibitors
- Microplate reader capable of measuring absorbance at 450 nm
- Absorbent paper
- Distilled or deionized water

V. Storage and Handling:

The kit can be used within one year if the whole kit is stored at -20°C upon receipt. Avoid repeated freeze-thaw cycles. Storage condition for opened reagents are listed in section IV.

VI. Reagent and Sample Preparation:

Note: Bring all reagents and samples to room temperature before use. Thaw the positive control and samples and keep them on ice before adding into wells. Please note that the amount of total protein containing the target protein to be used in this test can be optimized and must be determined by the end users.

- **Transcription factor binding reaction system:** Prepare 100 µl transcription factor binding reaction system for each well with 5 x DNA Binding Buffer, ATF-ELISA assay Reagent, DTT, Specific Competitor DNA Probe, Non-specific Competitor DNA Probe, and Positive Control or samples containing targeted proteins. Typical examples are shown in the table below.
  Note: Each reaction may be prepared in a labeled microfuge tube or directly in the coated plate well. If the reaction system is prepared directly in the coated plate wells, please add the reagents sequentially as shown in the table to get the best results.

- **Primary antibody:** Briefly spin down the NRF2 Primary Antibody vial. Add 100 µl of Antibody Diluent Buffer into the vial to prepare a primary antibody concentrate. Pipette up and down to mix gently. The primary antibody concentrate should then be diluted 100-fold with the Antibody Diluent.
- **Secondary antibody**: Briefly spin down the HRP-conjugated Secondary Antibody vial before use. Add 100 µl of Antibody Diluent Buffer into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently. The detection antibody concentrate should then be diluted 100-fold with the Antibody Diluent Buffer.
- **Wash Buffer**: Dilute 25 ml of the 20x Wash Buffer Concentrate into deionized or distilled water to yield 500 ml of 1x Wash Buffer. If the Wash Buffer Concentrate (20x) contains visible crystals, warm to room temperature and mix gently until dissolved.

	REACTION				
COMPONENT	Positive control	Sample	Specific competitor	Non-Specific competitor	Blank
5x TF-Activity Assay DNA Binding Buffer	20 µl	20 µl	20 µl	20 µl	20 µl
TF-Activity Assay Reagent	1.5 µl	1.5 µl	1.5 µl	1.5 µl	1.5 µl
DTT	1 µl	1 µl	1 µl	1 µl	1 µl
Specific Competitor	-	-	10 µl	-	-
Non-specific Competitor	-	-	-	10 µl	-
Control/Sample containing proteins	5 µl	* µl	* µl	* µl	-
Total volume	bring final volume to **100µl** with deionized water	bring final volume to **100µl** with deionized water	bring final volume to **100µl** with deionized water	bring final volume to **100µl** with deionized water	bring final volume to **100µl** with deionized water

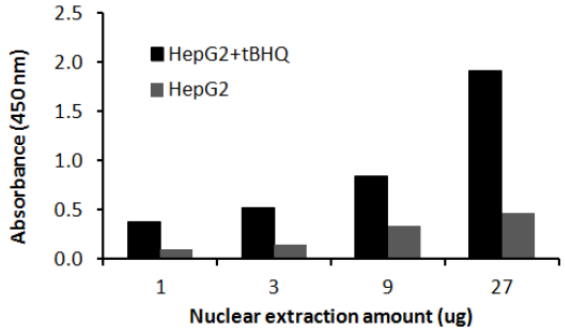
VII. Assay Protocol:

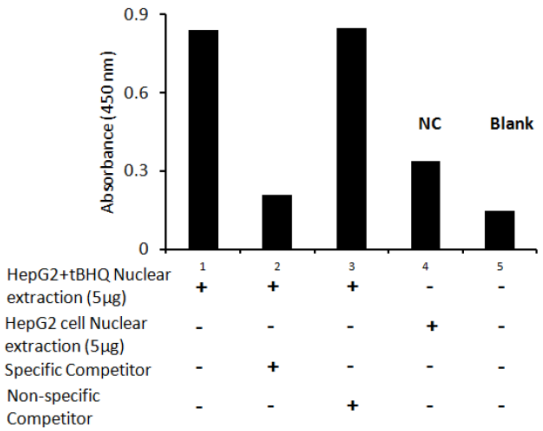
Note: Bring all reagents to room temperature (18 - 25°C) before use. A positive control should be included every time to confirm correct operation of experiment, however it is not necessary to run specific competitor and non-specific competitor for each sample and every time. It is recommended that all positive control and samples be run at least in duplicate.

1. Add 100 µl of each prepared **transcription factor binding reaction system** including **positive control, specific competitor, non-specific competitor** and **samples** into appropriate wells. Cover wells and incubate for 2 hours at room temperature or overnight at 4°C with gentle shaking.
2. Discard the solution and wash 4 times by filling each well with 300 µl of 1x **Wash Buffer**. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
3. Add 100 µl of prepared **NRF2 Primary Antibody** to each well. Incubate for 1 hour at room temperature with gentle shaking.
4. Discard the solution. Repeat the wash as in step 2.
5. Add 100 µl of prepared **HRP-conjugated Secondary Antibody** to each well. Incubate for 1 hour at room temperature with gentle shaking.
6. Discard the solution. Wash as directed in step 2.
7. Add 100 µl of **TMB One-Step Substrate Reagent** to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.
8. Add 50 µl of **Stop Solution** to each well. Read at 450 nm immediately.

VIII. Typical Data:

- (Left) Transcription factor activity assay of NRF2 from nuclear extracts of HepG2 cells or HepG2 cells treated with tBHQ (90uM) for 24 hr. After stimulation activated NRF2 is translocated into the nucleus where it binds with its corresponding DNA.
- (Right) Transcription factor activity assay of NRF2 from nuclear extracts of HepG2 cells or HepG2 cells treated with tBHQ (90uM) for 24 hr with the specific competitor or non-specific competitor. The result shows specific binding of NRF2 to the ARE binding site detected by using this kit.





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