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# Data Sheet

## *Keap1:Nrf2 Inhibitor Screening Assay Kit*

**Catalog #72020**

**DESCRIPTION:** The Keap1:Nrf2 Inhibitor Screening Assay Kit is designed for identification of inhibitors of Keap1:Nrf2 binding using fluorescence polarization. The kit comes in a convenient 96-well format, with purified Keap1 protein, a fluorescently labeled Nrf2 peptide containing the ETGE motif, and assay buffer for 100 protein reactions. Using this kit, only one simple step on a microtiter plate is required for Keap1:Nrf2 inhibitor screening reactions. The Keap1 protein and fluorescently labeled Nrf2 peptide are incubated with a sample containing testing inhibitors for 30 minutes. The change in fluorescent polarization can then be measured using a fluorescence reader.

**BACKGROUND:** The nuclear factor erythroid 2-related factor (Nrf2) is a transcription factor that induces the expression of antioxidant and phase II proteins by binding to the ARE (antioxidant response element) region of the gene promoter. Under basal conditions, Nrf2 is retained in the cytosol by binding to the cytoskeletal protein Keap1. Upon exposure to oxidative stress and other ARE activators, Nrf2 is released from Keap1. It then translocates to the nucleus, where it can bind to the ARE and activate expression of antioxidant and phase II proteins that protect the cell from oxidative damage. Because Nrf2 activation leads to a coordinated antioxidant and anti-inflammatory response, and Keap1 represses Nrf2 activation, Keap1 has become a very attractive drug target.

**COMPONENTS:**

| *Catalog #* | Component | Amount | Storage | Warnings |
|---|---|---|---|---|
| 70040 | Keap1, human recombinant | 30 µg | -80°C | Avoid freeze/thaw cycles. Protect FAM-Nrf2 from light. |
| 79112 | FAM-Nrf2 peptide (10 µM) | 10 µl | -80°C | |
| 79113 | Keap1-Nrf2 Assay Buffer | 50 ml | -20°C | |
| | BSA, 10 mg/ml | 100 µl | -80°C | |
| 79685 | 96-well black plate | 1 | Room Temp. | |

**APPLICATIONS:** Great for screening small molecular inhibitors for drug discovery and HTS applications.

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**MATERIALS REQUIRED BUT NOT SUPPLIED:**
Plate reader capable of fluorescence polarization measurements, λex = 475-495 nm, λem = 518-538 nm.
Adjustable micropipettor and sterile tips

**REFERENCE:** Inoyama, D., *et al.* 2012. *J. Biomol. Screening* **17(4):** 435-447.

**ASSAY PROTOCOL:**
All samples and controls should be tested in duplicate or triplicate.

1) Thaw **Nrf2 peptide** on ice. Upon first thaw, briefly spin tube containing protein to recover the full contents of the tube. Aliquot **Nrf2 peptide** into single-use aliquots. Store remaining undiluted **Nrf2 peptide** in aliquots at −80°C immediately.

2) Dilute Nrf2 peptide 1:10 in **Assay Buffer**. Keep diluted peptide on ice.

3) Prepare the master mixture: N wells × (24 µl **Assay Buffer** + 0.5 µl **BSA (10 mg/ml)** + 0.5 µl diluted **Nrf2 peptide**). Add 25 µl to each well labeled "Test Inhibitor, "Nrf2 Positive Binding Control", and "Nrf2 Negative Binding Control". For the wells labeled "Blank", add 45 µl of **Assay Buffer**.

4) Add 5 µl of inhibitor solution to each well designated "Test Inhibitor". Add 5 µl of the same solution without inhibitor (inhibitor buffer) to the wells labeled "Nrf2 Positive Binding Control", "Nrf2 Negative Binding Control", and "Blank".

| | **"Blank" Negative Control** | **Nrf2 Negative Binding Control** | **Nrf2 Positive Binding Control** | **Test Inhibitor** |
|---|---|---|---|---|
| Binding assay buffer | 45 µl | 44 µl | 24 µl | 24 µl |
| BSA (10 mg/ml) | − | 0.5 µl | 0.5 µl | 0.5 µl |
| Nrf2 peptide (1 µM) | − | 0.5 µl | 0.5 µl | 0.5 µl |
| Inhibitor | − | − | − | 5 µl |
| Inhibitor buffer (no inhibitor) | 5 µl | 5 µl | 5 µl | − |
| Keap1 (15 ng/µl) | − | − | 20 µl | 20 µl |
| **Total** | **50 µl** | **50 µl** | **50 µl** | **50 µl** |

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5) Thaw **Keap1** protein on ice. Upon first thaw, briefly spin tube containing protein to recover the full contents of the tube. Aliquot **Keap1** into single-use aliquots. Store remaining undiluted **Keap1** in aliquots at −80°C immediately. *Note:* **Keap1** *is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted protein.*

6) Dilute **Keap1** in assay buffer to 15 ng/µl (300 ng/reaction)*. Keep diluted protein on ice. Discard any remaining diluted protein after use. **Note: optimal protein concentration may vary.*

7) Add 20 µl of **Assay Buffer** to each well designated "Nrf2 Negative Binding Control".

8) Initiate binding reaction by adding 20 µl of diluted **Keap1** to wells designated "Nrf2 Positive Binding Control" and "Test Inhibitor." Incubate at room temperature for 30 minutes.

9) Read the fluorescent polarization of the sample in a microtiter-plate reader capable of excitation at wavelengths ranging from 475-495 nm and detection of emitted light ranging from 518-538 nm. Blank value is subtracted from all other values.

**CALCULATING RESULTS:**

**Definition of Fluorescence Polarization:**

$$P = \frac{I_{II} - I_{\perp}}{I_{II} + I_{\perp}}$$

where $I_{\parallel}$ = Intensity with polarizers parallel and $I_{\perp}$= Intensity with polarizers perpendicular. Most instruments display fluorescence polarization in units of mP.

$$mP = \left(\frac{I_{II} - I_{\perp}}{I_{II} + I_{\perp}}\right) x\ 1000$$

The equation above assumes that light is transmitted equally well through both parallel and perpendicular oriented polarizers. In practice, this is generally not true and a correction must be made to measure the absolute polarization state of the molecule. This correction factor is called the "G Factor".

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$$mP = \left(\frac{\mathrm{I}_{II} - G(\mathrm{I}_{\perp})}{\mathrm{I}_{II} + G(\mathrm{I}_{\perp})}\right) x\ 1000 \qquad \textbf{OR} \qquad mP = \left(\frac{G(\mathrm{I}_{II}) - \mathrm{I}_{\perp}}{G(\mathrm{I}_{II}) + \mathrm{I}_{\perp}}\right) x\ 1000$$

The G-factor is instrument-dependent and may vary slightly depending upon instrument and conditions. Please check the manual of your instrument to obtain the information about the establishment of the G-factor.

**EXAMPLE OF ASSAY RESULTS:**

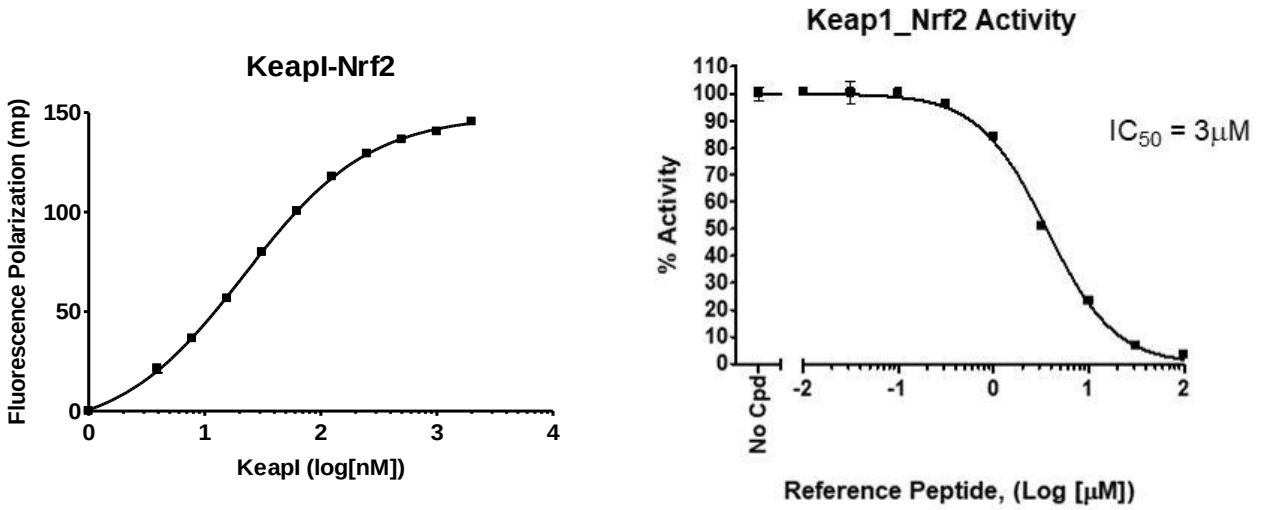


**Figure Legend:** Binding of Keap1 to Nrf2 (left) and inhibition of Keap1:Nrf2 binding by Nrf2 peptide inhibitor (right), measured using the Keap1:Nrf2 Inhibitor Screening Assay Kit, BPS Bioscience # 72020. Fluorescence was measured at λex 485nm, λem 530 nm using a Bio-Tek fluorescent microplate reader. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

**RELATED PRODUCTS:**

| **Product** | **Cat. #** | **Size** |
|---|---|---|
| ARE Reporter Kit (Nrf2 Antioxidant Pathway ) | 60514 | 500 reactions |
| ARE reporter cell line (Nrf2 Antioxidant pathway) | 60513 | 2 vials |
| Keap1 protein | 70040 | 100 µg |