Nrf2 Transcription Factor Assay Kit

Item No. 600590

Customer Service 800.364.9897 * **Technical Support** 888.526.5351
www.caymanchem.com

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Materials Supplied

Kit components may be stored at -20°C prior to use. For long term storage, the Positive Control should be thawed on ice, aliquoted at 75 µl/vial, and stored at -80°C. After opening the kit, we recommend each kit component be stored according to the temperature listed below.

Item Number	Item	Quantity	Storage
10006880	Transcription Factor Binding Assay Buffer (4X)	1 vial/3 ml	4°C
10007472	Transcription Factor Reagent A	1 vial/120 µl	-20°C
600591	Transcription Factor Nrf2 Positive Control	1 vial/500 µl	-80°C
10006882	Transcription Factor Antibody Binding Buffer (10X)	1 vial/3 ml	4°C
600592	Transcription Factor Nrf2 Primary Antibody	1 vial/120 µl	-20°C
400062	Wash Buffer Concentrate (400X)	1 vial/5 ml	RT
400035	Polysorbate 20	1 vial/3 ml	RT
600593	Transcription Factor Nrf2 Competitor dsDNA	1 vial/120 µl	-20°C
10006884	Transcription Factor Goat Anti-Rabbit HRP Conjugate	1 vial/120 µl	-20°C
600594	Transcription Factor Nrf2 96-Well Strip Plate	1 plate	4°C
400012	96-Well Cover Sheet	1 cover	RT
10006888	Transcription Factor Developing Solution	1 vial/12 ml	4°C
10006889	Transcription Factor Stop Solution	1 vial/12 ml	RT

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: This product is for laboratory research use only: not for administration to humans. Not for human or veterinary diagnostic or therapeutic use.

Precautions

Please read these instructions carefully before beginning this assay.

Kit components may be stored at -20°C prior to use. For long term storage, the Positive Control should be thawed on ice, aliquoted at 75 µl/vial and stored at -80°C. If the assay will be used on multiple days, we recommend each kit component be stored according to the temperatures listed in the booklet.

For research use only. Not for human or diagnostic use.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Fax: 734-971-3641

E-Mail: techserv@caymanchem.com

Hours: M-F 8:00 AM to 5:30 PM EST

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as directed and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. A plate reader capable of measuring absorbance at 450 nm
2. Adjustable pipettes and a repeating pipettor
3. A source of UltraPure water; glass Milli-Q or HPLC-grade water are acceptable
4. 300 mM Dithiothreitol (DTT)
5. Nuclear Extraction Kit available from Cayman (Item No. 10009277) or buffers for preparation of nuclear extracts (see pages 8-10)

NOTE: The components in each kit lot have been quality assured and warranted in this specific combination only; please do not mix them with components from other lots.

INTRODUCTION

Background

Nuclear factor E2-related factor 2 (Nrf2) is a leucine zipper-type transcription factor that is ubiquitously expressed with highest concentrations in the kidney, muscle, lung, and liver. Nrf2 plays a key role in maintaining redox homeostasis, *via* its interaction with a cysteine-rich protein Kelch-like ECH-associated protein 1 (Keap1).[1] In resting cells, Nrf2 and Keap1 form a tight complex which is targeted for degradation by proteasomes. Under oxidative stress, Keap1 may undergo a conformational change or Nrf2 may be phosphorylated; both lead to release of Nrf2 from the Nrf2/Keap1 complex and translocation of Nrf2 to the nucleus. Translocation results in induced expression of a battery of genes encoding diverse cytoprotective proteins, including antioxidative enzymes, anti-inflammatory mediators, and proteasomes.[2] These antioxidative enzymes activate a cell defense system which protects cells from molecular damage, thus lowering the risk of cancer development.

Recent studies reveal that Nrf2 is involved in many diseases, including cancer, diabetes, and neurodegenerative diseases. Dietary factors such as curcumin have been shown to activate Nrf2 and its downstream targets, which in turn lowers inflammation and inhibits carcinogensis.[3] The central role that Nrf2 plays in the protection of cells against oxidative and xenobiotic damage makes it a potential drug target in the intervention of disorders such as inflammation, neurodegeneration, and cancer.[4,5]

About This Assay

Cayman's Nrf2 Transcription Factor Assay is a non-radioactive, sensitive method for detecting specific transcription factor DNA binding activity in nuclear extracts. A 96-well enzyme-linked immunosorbent assay (ELISA) replaces the cumbersome radioactive electrophoretic mobility shift assay (EMSA). A specific double stranded DNA (dsDNA) sequence containing the Nrf2 response element is immobilized onto the wells of a 96-well plate (see Figure 1 on page 7). Nrf2 contained in a nuclear extract, binds specifically to the Nrf2 response element. Nrf2 is detected by addition of a specific primary antibody directed against Nrf2. A secondary antibody conjugated to HRP is added to provide a sensitive colorimetric readout at 450 nm.

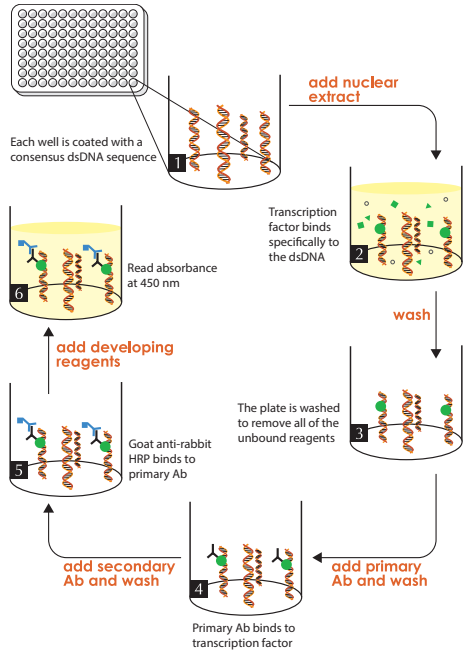


Figure 1. Schematic of the Transcription Factor Binding Assay

PRE-ASSAY PREPARATION

Sample Buffer Preparation

All buffers and reagents below required for preparation of Nuclear Extracts can be purchased directly from Cayman. Item numbers for each item are in the **Related Products** section on page 25. Alternatively, Cayman's Nuclear Extraction Kit (Item No. 10009277) can be used to isolate Nuclear Proteins.

1. Nuclear Extraction PBS (10X)

1.71 M NaCl, 33.53 mM KCl, 126.8 mM Na_2HPO_4, 22.04 mM KH_2PO_4, pH 7.4

2. Nuclear Extraction PBS (1X)

Dilute 100 ml of 10X stock with 900 ml distilled H_2O

3. Nuclear Extraction Phosphatase Inhibitor Cocktail (50X)

0.5 M NaF

0.05 M β-glycerophosphate

0.05 M Na_3OV_4

Store at -80°C

4. Nuclear Extraction PBS/Phosphatase Inhibitor Solution (1X)

Add 200 µl of 50X Phosphatase Inhibitor Solution to 10 ml of 1X Nuclear Extraction PBS, mix well, and keep on ice. Make fresh daily.

5. Nuclear Extraction Protease Inhibitor Cocktail (100X)

10 mM AEBSF

0.5 mM Bestatin

0.2 mM Leupeptin Hemisulfate Salt

0.15 mM E-64

0.1 mM Pepstatin A

0.008 mM Aprotinin from Bovine Lung

Made in DMSO, store at -20°C

6. Nuclear Extraction Hypotonic Buffer (10X)

100 mM HEPES, pH 7.5, containing 40 mM NaF, 10 µM Na_2MoO_4, and 0.1 mM EDTA

Store at 4°C

7. Complete Hypotonic Buffer (1X)

Prepare as outlined in Table 1. The phosphatase and protease inhibitors lose activity shortly after dilution; therefore any unused 1X Complete Hypotonic Buffer should be discarded.

Reagent	150 mm plate ~1.5 x 10^7 cells
Hypotonic Buffer (10X)	100 µl
Phosphatase Inhibitors (50X)	20 µl
Protease Inhibitors (100X)	10 µl
Distilled Water	870 µl
Total Volume	1,000 µl

Table 1. Preparation of Complete Hypotonic Buffer

8. Nonidet P-40 Assay Reagent (10%)

Nonidet P-40 or suitable substitute at a concentration of 10% (v/v) in H_2O

Store at room temperature

9. Nuclear Extraction Buffer (2X)

20 mM HEPES, pH 7.9, containing, 0.2 mM EDTA, 3 mM $MgCl_2$, 840 mM NaCl, and 20% glycerol (v/v)

Store at 4°C

10. Complete Nuclear Extraction Buffer (1X)

Prepare as outlined in Table 2. Some of the phosphatase and protease inhibitors lose activity shortly after dilution; therefore any remaining 1X Extraction Buffer should be discarded.

Reagent	150 mm plate ~1.5 x 10^7 cells
Nuclear Extraction Buffer (2X)	75 µl
Protease Inhibitors (100X)	1.5 µl
Phosphatase Inhibitors (50X)	3.0 µl
DTT (10 mM)	15 µl
Distilled Water	55.5 µl
Total Volume	150 µl

Table 2. Preparation of Complete Nuclear Extraction Buffer

Purification of Cellular Nuclear Extracts

Cayman's Nuclear Extraction Kit (Item No. 10009277) can be used to isolate nuclear proteins. Alternatively, the procedure described below can be used for a 15 ml cell suspension grown in a T75 flask or adherent cells (100 mm dish 80-90% confluent) where 10^7 cells yields approximately 50 µg of nuclear protein.

1. Collect ~10^7 cells in pre-chilled 15 ml tubes.
2. Centrifuge suspended cells at 300 x g for five minutes at 4°C.
3. Discard the supernatant. Resuspend cell pellet in 5 ml of ice-cold 1X Nuclear Extraction PBS/Phosphatase Inhibitor Solution and centrifuge at 300 x g for five minutes at 4°C. Repeat one time.
4. Discard the supernatant. Add 500 µl ice-cold 1X Complete Hypotonic Buffer. Mix gently by pipetting and transfer resuspended pellet to pre-chilled 1.5 ml microcentrifuge tube.
5. Incubate cells on ice for 15 minutes allowing cells to swell.
6. Add 100 µl of 10% Nonidet P-40 (or suitable substitute). Mix gently by pipetting.
7. Centrifuge for 30 seconds (pulse spin) at 4°C in a microcentrifuge. Transfer the supernatant which contains the cytosolic fraction to a new tube and store at -80°C.
8. Resuspend the pellet in 100 µl ice-cold Complete Nuclear Extraction Buffer (1X) (with protease and phosphatase inhibitors). Vortex 15 seconds at highest setting then gently rock the tube on ice for 15 minutes using a shaking platform. Vortex sample for 30 seconds at highest setting and gently rock for an additional 15 minutes.
9. Centrifuge at 14,000 x g for 10 minutes at 4°C. The supernatant contains the nuclear fraction. Aliquot to clean chilled tubes, flash freeze, and store at -80°C. Avoid freeze/thaw cycles. The extracts are ready to use in the assay.
10. Keep a small aliquot of the nuclear extract to quantitate the protein concentration.

Reagent Preparation

1. Transcription Factor Antibody Binding Buffer (10X)

One vial (Item No. 10006882) contains 3 ml of a 10X stock of Transcription Factor Antibody Binding Buffer (ABB) to be used for diluting the primary and secondary antibodies. To prepare a 1X ABB, dilute 1:10 by adding 27 ml of UltraPure water. Store at 4°C for up to six months.

2. Wash Buffer Concentrate (400X)

One vial (Item No. 400062) contains 5 ml of 400X Wash Buffer. Dilute the contents of the vial to a total volume of 2 liters with UltraPure water and add 1 ml of Polysorbate 20 (Item No. 400035). *NOTE: Polysorbate 20 is a viscous liquid and cannot be measured by a pipette. A positive displacement device such as a syringe should be used to deliver small quantities accurately.* A smaller volume of Wash Buffer Concentrate can be prepared by diluting the Wash Buffer Concentrate 1:400 and adding Polysorbate 20 (0.5 ml/liter of Wash Buffer). Store at 4°C for up to two months.

3. Transcription Factor Binding Assay Buffer (4X)

One vial (Item No. 10006880) contains 3 ml of a 4X stock of Transcription Factor Binding Assay Buffer (TFB). Prepare Complete Transcription Factor Binding Assay Buffer (CTFB) immediately prior to use in 1.5 ml centrifuge tubes or 15 ml conical tubes as outlined in Table 3, on page 13. This buffer is now referred to as CTFB. *It is recommended that the CTFB be used the same day it is prepared.*

Component	Volume/Well	Volume/Strip	Volume/ 96-well plate
UltraPure water	73 µl	584 µl	7,008 µl
4X Transcription Factor Binding Assay Buffer	25 µl	200 µl	2,400 µl
Reagent A (Item No. 10007472)	1 µl	8 µl	96 µl
300 mM DTT	1 µl	8 µl	96 µl
Total Required	100 µl	800 µl	9,600 µl

Table 3. Preparation of Complete Transcription Factor Binding Assay Buffer

4. Transcription Factor Nrf2 Positive Control

One vial (Item No. 600591) contains 500 µl of nuclear extract of HepG2 cells treated with 90 µM tert-butylhydroquinone for 24 hours. This nuclear extract solution is provided as a positive control for Nrf2 activation; it is not intended for plate to plate comparisons. The protein solution provided is sufficient for 20 reactions and will provide a strong signal (>0.5 AU at 450 nm) when used at 25 µl/well. When using this Positive Control, a decrease in signal may occur with repeated freeze/thaw cycles. It is recommended that the nuclear extract be aliquoted at 75 µl per vial and stored at -80°C to avoid loss in signal from repeated freeze/thaw cycles.

ASSAY PROTOCOL

Plate Set Up

There is no specific pattern for using the wells on the plate. A typical layout of Nrf2 Positive Control (PC), Competitor dsDNA (C1), and samples of nuclear extracts (S1-S44) to be measured in duplicate is given below in Figure 2. We suggest you record the contents of each well on the template sheet provided (see page 27).

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S1	S9	S9	S17	S17	S25	S25	S33	S33	S41	S41
B	S2	S2	S10	S10	S18	S18	S26	S26	S34	S34	S42	S42
C	S3	S3	S11	S11	S19	S19	S27	S27	S35	S35	S43	S43
D	S4	S4	S12	S12	S20	S20	S28	S28	S36	S36	S44	S44
E	S5	S5	S13	S13	S21	S21	S29	S29	S37	S37	NSB	NSB
F	S6	S6	S14	S14	S22	S22	S30	S30	S38	S38	PC	PC
G	S7	S7	S15	S15	S23	S23	S31	S31	S39	S39	Blk	Blk
H	S8	S8	S16	S16	S24	S24	S32	S32	S40	S40	C1	C1

S1-S44 - Sample Wells
NSB - Non-specific Binding Wells
PC - Positive Control Wells
Blk - Blank Wells
C1 - Competitor dsDNA Wells

Figure 2. Sample plate format

Pipetting Hints

- Use different tips to pipette each reagent.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (*i.e.*, slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

General Information

- It is not necessary to use all the wells on the plate at one time; however a Positive Control should be run every time.
- For each plate or set of strips it is recommended that two Blk, two NSB, and two PC wells be included.

Performing the Assay

Binding of active Nrf2 to the consensus sequence

1. Equilibrate the plate and buffers to room temperature prior to opening. Remove the plate from the foil and select the number of strips needed. The 96-well plate supplied with this kit is ready to use.

   NOTE: If you are not using all of the strips at once, place the unused strips back in the plate packet and store at 2-4°C. Be sure that the packet is sealed with the desiccant inside.

2. Prepare the CTFB as outlined in Table 3, on page 13.

3. Add appropriate amount of reagent(s) listed below to the designated wells as follows:

   Blk - add 100 µl of CTFB to designated wells.

   NSB - add 100 µl of CTFB to designated wells. Do not add samples or Positive Control to these wells.

   C1 - Add 80 µl of CTFB prior to adding 10 µl of Transcription Factor Nrf2 Competitor dsDNA (Item No. 600024) to designated wells. Add 10 µl of control cell lysate, or unknown sample.

   NOTE: Competitor dsDNA must be added prior to adding the Positive Control or nuclear extracts.

   S1-S44 - Add 90 µl of CTFB followed by 10 µl of Nuclear Extract to designated wells. A protocol for isolation of nuclear extracts is given on page 12.

   PC - Add 75 µl of CTFB followed by 25 µl of Positive Control to appropriate wells.

4. Use the cover provided to seal the plate. Incubate overnight at 4°C or one hour at room temperature without agitation (incubation for one hour will result in a less sensitive assay).

5. Empty the wells and wash five times with 200 µl of 1X Wash Buffer. After each wash empty the wells in the sink. After the final wash (wash #5), tap the plate on a paper towel to remove any residual Wash Buffer.

Addition of Transcription Factor Nrf2 Primary Antibody

1. Dilute the Transcription Factor Nrf2 Primary Antibody (Item No. 600592) 1:100 in 1X ABB as outlined in Table 4 below. Add 100 µl of diluted Nrf2 Primary Antibody to each well except the Blk wells.

Component	Volume/Well	Volume/Strip	Volume/ 96-well plate
1X ABB	99 µl	792 µl	9,504 µl
Nrf2 Primary Antibody	1 µl	8 µl	96 µl
Total required	100 µl	800 µl	9,600 µl

Table 4. Dilution of Primary Antibody

2. Use the adhesive cover provided to seal the plate.

3. Incubate the plate for one hour at room temperature without agitation.

4. Empty the wells and wash each well five times with 200 µl of 1X Wash Buffer. After each wash, empty the contents of the plate into the sink. After the final wash (wash #5), tap the plate three to five times on a paper towel to remove any residual Wash Buffer.

Addition of the Transcription Factor Goat Anti-Rabbit HRP Conjugate

1. Dilute the Transcription Factor Goat Anti-Rabbit HRP Conjugate (Item No. 10006884) 1:100 in 1X ABB as outlined in Table 5 below. Add 100 µl of diluted secondary antibody to each well except the Blk wells.

Component	Volume/Well	Volume/Strip	Volume/ 96-well plate
1X ABB	99 µl	792 µl	9,504 µl
Goat Anti-Rabbit HRP Conjugate	1 µl	8 µl	96 µl
Total required	100 µl	800 µl	9,600 µl

Table 5. Dilution of Secondary Antibody

2. Use the adhesive cover provided to seal the plate.

3. Incubate for one hour at room temperature without agitation.

4. Empty the wells and wash five times with 200 µl of 1X Wash Buffer. After each wash, empty the contents of the plate into the sink. After the final wash (wash #5), tap the plate three to five times on a paper towel to remove any residual Wash Buffer.

Develop and Read the Plate

1. To each well being used add 100 µl of Transcription Factor Developing Solution (Item No. 10006888), which has been equilibrated to room temperature.
2. Incubate the plate for 15 to 45 minutes at room temperature with gentle agitation protected from light. Allow the wells to turn medium to dark blue prior to adding Transcription Factor Stop Solution (Item No. 10006889). (This reaction can be monitored by taking absorbance measurements at 655 nm prior to stopping the reactions; An OD_{655} of 0.4-0.5 yields an OD_{450} of approximately 1). Monitor development of sample wells to ensure adequate color development prior to stopping the reaction. *NOTE: Do not overdevelop; however PC wells may need to overdevelop to allow adequate color development in sample wells.*
3. Add 100 µl of Stop Solution per well being used. The solution within the wells will change from blue to yellow after adding the Stop Solution.
4. Read absorbance at 450 nm within five minutes of adding the Stop Solution. Blank the plate reader according to the manufacturer's requirements using the blank wells.

Assay Procedure Summary

NOTE: This procedure is provided as a quick reference for experienced users. Follow the detailed procedure when initially performing the assay.

1. Prepare CTFB as described in the **Pre-Assay Preparation** section, Table 3 on page 13.
2. Add 90 µl CTFB per sample well (80 µl if adding Competitor dsDNA), 100 µl to Blk and NSB wells).
3. Add 10 µl of Competitor dsDNA (optional) to appropriate wells.
4. Add 25 µl of Positive Control to appropriate wells.
5. Add 10 µl of Sample containing Nrf2 to appropriate wells.
6. Incubate overnight at 4°C or one hour at room temperature without agitation.
7. Wash each well five times with 200 µl of 1X Wash Buffer.
8. Add 100 µl of diluted Nrf2 Primary Antibody per well (except Blk wells).
9. Incubate one hour at room temperature without agitation.
10. Wash each well five times with 200 µl of 1X Wash Buffer.
11. Add 100 µl of diluted Secondary Antibody (except Blk wells).
12. Incubate one hour at room temperature without agitation.
13. Wash each well five times with 200 µl of 1X Wash Buffer.
14. Add 100 µl of Developing Solution per well.
15. Incubate 15 to 45 minutes with gentle agitation.
16. Add 100 µl of Stop Solution per well.
17. Measure the absorbance at 450 nm.

Steps	Reagent	Blk	NSB	PC	C1	S1-S44
1. Add reagents	CTFB	100 µl	100 µl	75 µl	65 µl	90 µl
	Competitor dsDNA				10 µl	
	Positive Control			25 µl	25 µl	
	Samples					10 µl
2. Incubate	Cover plate and incubate overnight at 4°C or one hour at room temperature without agitation					
3. Wash	Wash all wells five times					
4. Add reagents	Primary Antibody		100 µl	100 µl	100 µl	100 µl
5. Incubate	Cover plate and incubate one hour at room temperature without agitation					
6. Wash	Wash all wells five times					
7. Add reagents	Secondary Antibody		100 µl	100 µl	100 µl	100 µl
8. Incubate	Cover plate and incubate one hour at room temperature without agitation					
9. Wash	Wash all wells five times					
10. Add reagents	Developer Solution	100 µl	100 µl	100 µl	100 µl	100 µl
11. Incubate	Monitor development in wells					
12. Add reagents	Stop Solution	100 µl	100 µl	100 µl	100 µl	100 µl
13. Read	Read plate at wavelength of 450 nm					

Table 6. Quick Protocol Guide

ANALYSIS

Performance Characteristics

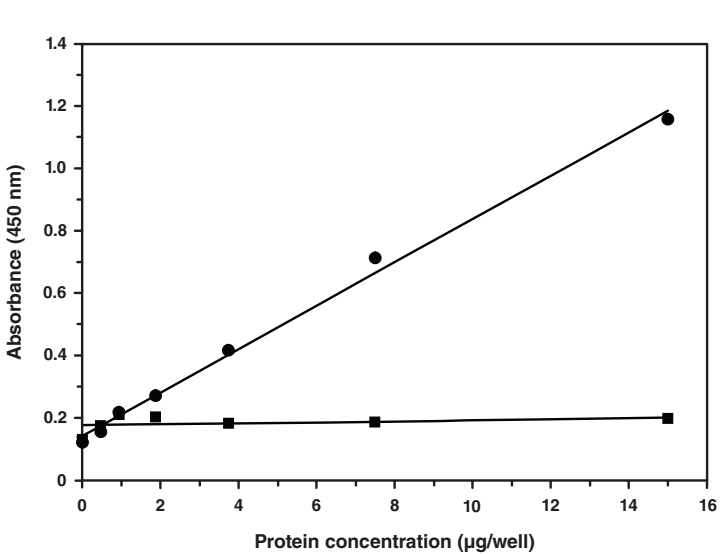


Figure 3. Assay of Nrf2 in a nuclear extract from HepG2 cells treated with 90 µM tert-butylhydroquinone for 24 hours

RESOURCES

Interferences

The following reagents were tested for interference in the assay.

Reagent	Will Interfere (Yes or No)
EGTA (≤1 mM)	No
EDTA (≤0.5 mM)	No
ZnCl (any concentration)	Yes
DTT (between 1 and 5 mM)	No
Dimethylsulfoxide (≤1.5%)	No

Troubleshooting

Problem	Possible Causes	Recommended Solutions
No signal or weak signal in all wells	A. Omission of key reagent B. Plate reader settings not correct C. Reagent/reagents expired D. Salt concentrations affected binding between DNA and protein E. Developing reagent used cold F. Developing reagent not added to correct volume	A. Check that all reagents have been added and in the correct order; perform the assay using the Positive Control B. Check wavelength setting on plate reader and change to 450 nm C. Check expiration date on reagents D. Reduce the amount of nuclear extract used in the assay, or reduce the amount of salt in the nuclear extracts (alternatively can perform buffer exchange) E. Prewarm the Developing Solution to room temperature prior to use F. Check pipettes to ensure correct amount of Developing Solution was added to wells
High signal in all wells	A. Incorrect dilution of antibody (too high) B. Improper/inadequate washing of wells C. Over-developing	A. Check antibody dilutions and use amounts outlined in instructions B. Follow the protocol for washing wells using the correct number of times and volumes C. Decrease the incubation time when using the developing reagent
High background (NSB)	Incorrect dilution of antibody (too high)	Check antibody dilutions and use amounts outlined in the instructions

Problem (cont.)	Possible Causes (cont.)	Recommended Solutions (cont.)
Weak signal in sample wells	A. Sample concentration is too low B. Incorrect dilution of antibody C. Salt concentrations affecting binding between DNA and protein	A. Increase the amount of nuclear extract used; loss of signal can occur with multiple freeze/thaw cycles of the sample; prepare fresh nuclear extracts and aliquot as outlined in product insert B. Check antibody dilutions and use amounts outlined in the instructions C. Reduce the amount of nuclear extract used in the assay or reduce the amount of salt in the nuclear extracts (alternatively can perform buffer exchange)

References

1. Surh, Y.-J., Kundu, J.K., and Na, H.-K. Nrf2 as a master redox switch in turning on the cellular signaling involved in the induction of cytoprotective genes by some chemopreventive phytochemicals. *Planta Med.* **74**, 1526-1539 (2008).
2. Taguchi, K., Motohashi, H., and Yamamoto, M. Molecular mechanisms of the Keap1-Nrf2 pathway in stress response and cancer evolution. *Genes Cells* **16**, 123-140 (2011).
3. Martín-Montalvo, A., Villalba, J.M., Navas, P., *et al.* Nrf2, cancer and calorie restriction. *Oncogene* **30**, 505-520 (2011).
4. Tufekci, K.U., Bayin, E.C., Genc, S., *et al.* The Nrf2/ARE pathway: A promising target to counteract mitochondrial dysfunction in Parkinson's disease. *Parkinsons Dis.* 1-14 (2011).
5. Surh, Y.-J. and Na, H.-K. NF-κB and Nrf2 as prime molecular targets for chemoprevention and cytoprotection with anti-inflammatory and antioxidant phytochemicals. *Genes Nutr.* **2**, 313-317 (2008).

Related Products

ATF2 (Phospho-Tyr$^{69/71}$) Transcription Factor Assay Kit - Item No. 600130
ChREBP Transcription Factor Assay Kit - Item No. 10006909
CREB (Phospho-Ser^{133}) Transcription Factor Assay Kit - Item No. 10009846
HIF-1α Transcription Factor Assay Kit - Item No. 10006910
Liver X Receptor β Transcription Factor Assay Kit - Item No. 10011119
NF-κB (human p50) Transcription Factor Assay Kit - Item No. 10006912
NF-κB (human p50/p65) Combo Transcription Factor Assay Kit - Item No. 10011223
NF-κB p50 (human recombinant) - Item No. 10009818
NF-κB (p65) Transcription Factor Assay Kit - Item No. 10007889
Nonidet P-40 Assay Reagent (10%) - Item No. 600009
Nrf2 (C-Term) Polyclonal Antibody - Item No. 10214
Nrf2 (N-Term) Polyclonal Antibody - Item No. 14114
Nuclear Extraction Buffer (2X) - Item No. 10009306
Nuclear Extraction Hypotonic Buffer (10X) - Item No. 10009301
Nuclear Extraction Kit - Item No. 10009277
Nuclear Extraction PBS (10X) - Item No. 10009304
Nuclear Extraction Phosphatase Inhibitors (50X) - Item No. 10009305
Nuclear Extraction Protease Inhibitor Cocktail (100X) - Item No. 10009303
p53 Transcription Factor Assay Kit - Item No. 600020
p53 Designer Transcription Factor Assay Kit - Item No. 600030
PPARα, δ, γ Complete Transcription Factor Assay Kit - Item No. 10008878
PPARα Transcription Factor Assay Kit - Item No. 10006915
PPARδ Transcription Factor Assay Kit - Item No. 10006914
PPARγ Transcription Factor Assay Kit - Item No. 10006855
SREBP-1 Transcription Factor Assay Kit - Item No. 10010854
SREBP-2 Transcription Factor Assay Kit - Item No. 10007819

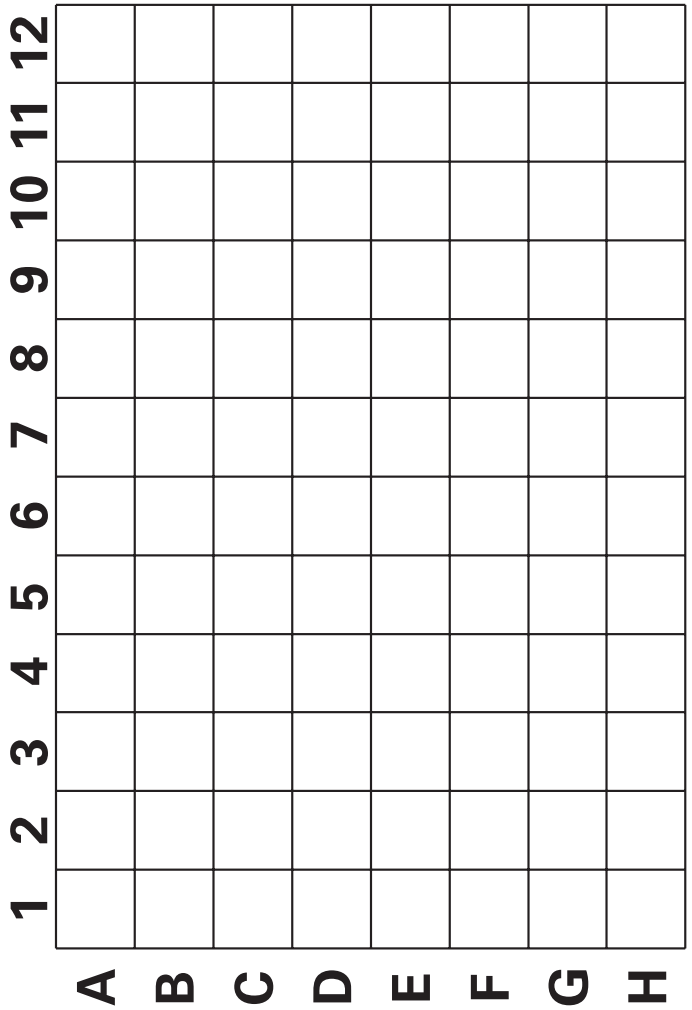
Warranty and Limitation of Remedy

Cayman Chemical Company makes **no warranty or guarantee** of any kind, whether written or oral, expressed or implied, including without limitation, any warranty of fitness for a particular purpose, suitability and merchantability, which extends beyond the description of the chemicals hereof. Cayman **warrants only** to the original customer that the material will meet our specifications at the time of delivery. Cayman will carry out its delivery obligations with due care and skill. Thus, in no event will Cayman have **any obligation or liability**, whether in tort (including negligence) or in contract, for any direct, indirect, incidental or consequential damages, even if Cayman is informed about their possible existence. This limitation of liability does not apply in the case of intentional acts or negligence of Cayman, its directors or its employees.

Buyer's **exclusive remedy** and Cayman's sole liability hereunder shall be limited to a refund of the purchase price, or at Cayman's option, the replacement, at no cost to Buyer, of all material that does not meet our specifications.

Said refund or replacement is conditioned on Buyer giving written notice to Cayman within thirty (30) days after arrival of the material at its destination. Failure of Buyer to give said notice within thirty (30) days shall constitute a waiver by Buyer of all claims hereunder with respect to said material.

For further details, please refer to our Warranty and Limitation of Remedy located on our website and in our catalog.



NOTES