SREBF1 Transcription Factor Assay Kit

Catalog Number KA1377

96 assays

Version: 03

Intended for research use only

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Introduction

Background

SREBP (Sterol Regulatory Element-Binding Protein) transcription factors are members of the basic helix-loop-helix-leucine zipper family of transcription factors. SREBPs activate the expression of more than thirty genes that regulate the synthesis and uptake of cholesterol, fatty acids, triglycerides, and phospholipids.[1] In addition, SREBPs have also been shown to play critical roles in adipocyte differentiation and insulin-dependent gene expression. There are three known isoforms of SREBP: SREBP-1a, SREBP-1c, and SREBP-2. SREBP-1a and SREBP-1c are derived from the same gene, but they differ at the first exon due to alternative splicing.[2] SREBPs are synthesized as membrane-bound precursor proteins anchored on the rough endoplasmic reticulum. These SREBPs are bound to Scap (SREBP cleavage activating protein) and remain bound to the endoplasmic reticulum when sterol concentration is high. However, when sterol concentration is low, the SREBP-Scap complex exits the endoplasmic reticulum and enters the Golgi. In the Golgi, the SREBPs undergo a sequential proteolytic two-step cleavage process by S1P and S2P that release the NH2-terminal active domain from the membrane. These newly cleaved SREBPs contain a nuclear localization signal that binds directly to importin, allowing the SREBPs to enter the nucleus (nSREBPs).[3] In the nucleus, the SREBPs bind to SRE sequences, upregulating genes required for sterol synthesis and regulation.[4]

SREBP-1 acts primarily to activate genes in fatty acid synthesis. Nuclear SREBP-1 up-regulates gene expression of a group of target lipogenic enzymes such as acetyl CoA carboxylase, fatty acid synthase, and long chain fatty acid elongase.[5] In addition, SREBP-1 may also contribute to the regulation of glucose uptake and synthesis through induction of glucokinase, a key enzyme in glycolysis.[6] SREBP-1 has many important clinical implications in the treatment of many diseases including obesity, diabetes mellitus, insulin resistance, and non-alcoholic fatty liver disease (NAFLD).

Principle of the Assay

SREBF1 Transcription Factor Assay Kit 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 SREBP response element is immobilized onto the wells of a 96-well plate. SREBP-1 contained in a nuclear extract binds specifically to the SREBP response element. SREBP-1 is detected by addition of a specific primary antibody directed against SREBP-1. A secondary antibody conjugated to HRP is added to provide a sensitive colorimetric readout at 450 nm. SREBF1 Transcription Factor Assay Kit detects human, rat, and murine SREBP-1.

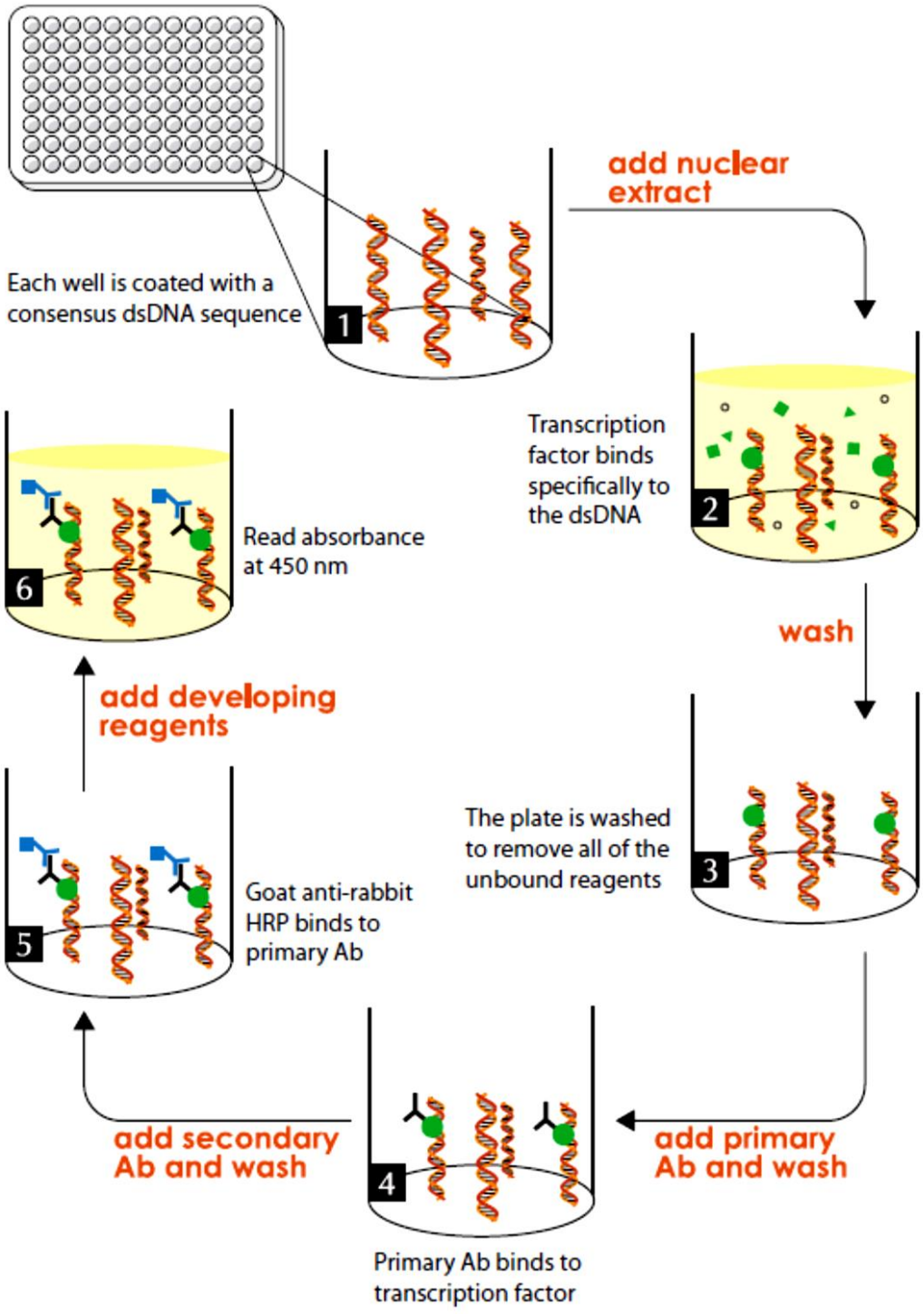

Figure 1. Schematic of the Transcription Factor Binding Assay

General Information

Materials Supplied

List of component

Item	Quantity/Sizes
Transcription Factor Binding Assay Buffer (4X)	1 vial/3 mL
Transcription Factor Reagent A	1 vial/120 µL
SREBP-1 Positive Control	1 vial/150 µL
Transcription Factor Antibody Binding Buffer (10X)	1 vial/3 mL
SREBP-1 Primary Antibody	1 vial/120 µL
Wash Buffer Concentrate (400X)	1 vial/5 mL
Polysorbate 20	1 vial/3 mL
Transcription Factor SREBP Competitor dsDNA	1 vial/120 µL
Transcription Factor Goat Anti-Rabbit HRP Conjugate	1 vial/120 µL
Transcription Factor SREBP 96-Well Strip Plate	1 plate
96-Well Cover Sheet	1 cover
Transcription Factor Developing Solution	1 vial/12 mL
Transcription Factor Stop Solution	1 vial/12 ml

Storage Instruction

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 25 µL/vial, and stored at -80°C. After use we recommend each kit component be stored according to the temperature listed below. This kit will perform as specified if stored as directed and used before the expiration date indicated on the outside of the box.

Item	Storage
Transcription Factor Binding Assay Buffer (4X)	4°C
Transcription Factor Reagent A	-20°C
SREBP-1 Positive Control	-80°C
Transcription Factor Antibody Binding Buffer (10X)	4°C
SREBP-1 Primary Antibody	-20°C
Wash Buffer Concentrate (400X)	RT
Polysorbate 20	RT
Transcription Factor SREBP Competitor dsDNA	-20°C
Transcription Factor Goat Anti-Rabbit HRP Conjugate	-20°C
Transcription Factor SREBP 96-Well Strip Plate	4°C
96-Well Cover Sheet	RT

Transcription Factor Developing Solution	4°C
Transcription Factor Stop Solution	RT

Materials Required but Not Supplied

- ✓ A plate reader capable of measuring absorbance at 450 nm
- ✓ Adjustable pipettes and a repeat pipettor
- ✓ A source of UltraPure water; glass Milli-Q or HPLC-grade water is acceptable
- ✓ 300 mM dithiothreitol (DTT)

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.

Precautions for Use

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

- 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 Blks, two Non-Specific Binding (NSB), and two PC wells be included.

- 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.

Assay Protocol

Reagent Preparation

- ✓ Transcription Factor Antibody Binding Buffer (10X) - One vial 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 1X ABB, dilute 1:10 by adding 27 mL of UltraPure water. Store at 4°C for up to six months.
- ✓ Wash Buffer Concentrate (400X) - One vial 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. *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 six months.
- ✓ Transcription Factor Binding Assay Buffer (4X) - One vial contains 3 mL of a 4X stock of Transcription Factor Binding Assay Buffer (TFB). Prepare Complete Transcription Factor Binding Buffer (CTFB) immediately prior to use in 1.5 mL centrifuge tubes or 15 mL conical tubes as outlined in Table 2. 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
Binding Assay Buffer (4X)	25 µL	200 µL	2,400 µL
Reagent A	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 1. Preparation of Complete Transcription Factor Binding Buffer

- ✓ Transcription Factor SREBP-1 Positive Control - One vial contains 150 µL of clarified cell lysate. This lysate is provided as a positive control for SREBP-1 activation; it is not intended for plate to plate comparisons. The Positive Control provided is sufficient for 15 reactions and will provide a strong signal (>0.5 AU at 450 nm) when used at 10 µL/well. When using this Positive Control, a decrease in signal may occur with repeated freeze/thaw cycles. It is recommended that the Positive Control be aliquoted at 25 µL per vial and stored at -80°C to avoid loss in signal from repeated freeze/thaw cycles.

Sample Preparation

All buffers and reagents below are used for preparation of Nuclear Extracts.

- ✓ 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
- ✓ Nuclear Extraction PBS (1X) - Dilute 100 mL of 10X stock with 900 mL distilled H_2O
- ✓ Nuclear Extraction Phosphatase Inhibitor Cocktail (50X)
  0.05 M NaF
  0.05 M β-glycerophosphate
  0.05 M Na_3OV_4
  Store at -80°C
- ✓ 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.
- ✓ 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
- ✓ Nuclear Extraction Hypotonic Buffer (10X) - 100 mM HEPES, pH 7.5, containing 40 mM NaF, 100 µM Na_2MoO_4, and 1 mM EDTA, Store at 4°C.
- ✓ Complete Hypotonic Buffer (1X) - Prepare as outlined in Table 2. 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 2. Preparation of Complete Hypotonic Buffer

- ✓ 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.
- ✓ 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.
- ✓ Complete Nuclear Extraction Buffer (1X) - Prepare as outlined in Table 3. 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 3. Preparation of Complete Nuclear Extraction Buffer

- Purification of Cellular Nuclear Extracts

  Nuclear Extraction Kit is recommended for preparation of nuclear extracts. Alternatively, the procedure 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 the cell pellet in 5 ml of ice-cold 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. 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 the highest setting and then gently rock the tube on ice for 15 minutes using a shaking platform. Vortex sample for 30 seconds at the 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.

Assay Procedure

- Performing the Assay

  Binding of active SREBP-1 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 4°C. Be sure that the packet is sealed with the desiccant inside.
2. Prepare the CTFB as outlined in Table 1.
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 SREBP Competitor dsDNA 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.

   PC - Add 90 µL of CTFB followed by 10 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 SREBP-1 Primary Antibody

1. Dilute the Transcription Factor SREBP-1 Primary Antibody 1:100 in 1X ABB as outlined in Table 4 below. Add 100 µL of diluted SREBP-1 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
SREBP-1 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 1:100 in 1X ABB as outlined in Table 5 below. Add 100 µL of diluted HRP Conjugated 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 HRP Conjugate

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. Add 100 µL of Transcription Factor Developing Solution, which has been equilibrated to room temperature, to each well being used.
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. (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 Positive Control 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.

- 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 Reagent Preparation section Table 1.
2. Add 90 µL CTFB per sample well (80 µL if adding Competitive dsDNA), 100 µL to Blk and NSB wells).
3. Add 10 µL of Competitive dsDNA (optional) to appropriate wells.
4. Add 10 µL of Positive Control to appropriate wells.
5. Add 10 µL of Sample containing SREBP-1 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 SREBP-1 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	90 µL	80 µL	90 µL
	Competitive dsDNA				10 µL	
	Positive Control			10 µL	10 µL	
	Samples					10 µL
2. Incubate	Cover plate and incubate overnight at 4°C 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	Goat Anti-Rabbit HRP Conjugate		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

Data Analysis

Performance Characteristics

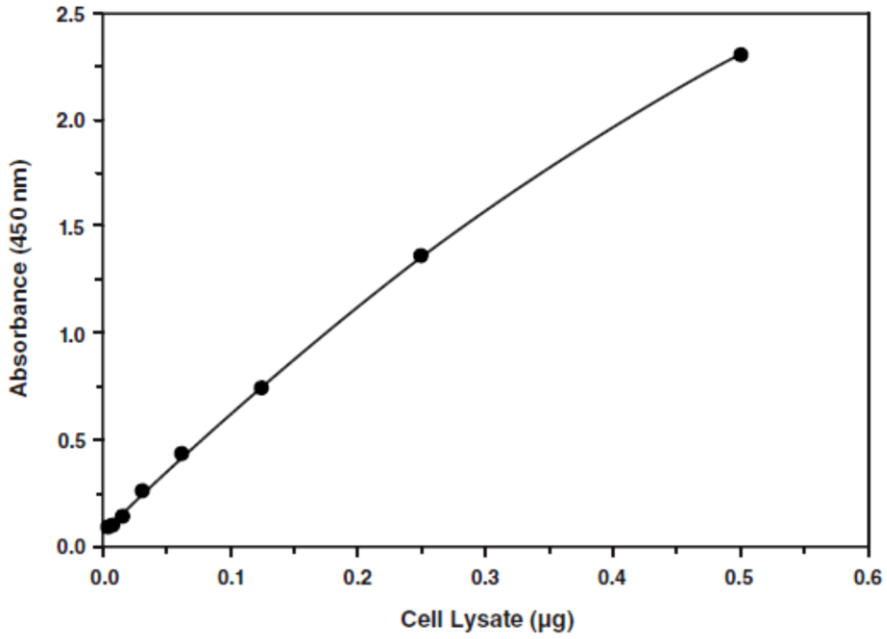

Figure 3. Assay of recombinant SREBP-1 from E. coli cell lysates

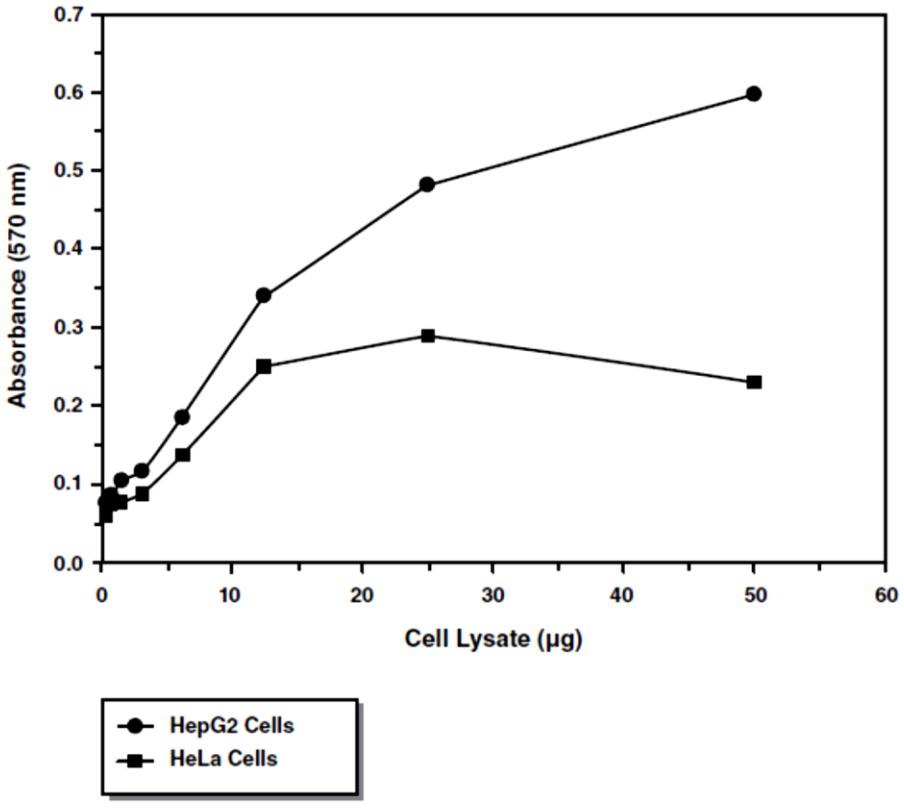

Figure 4. Assay of HeLa and HepG2 cell lysates

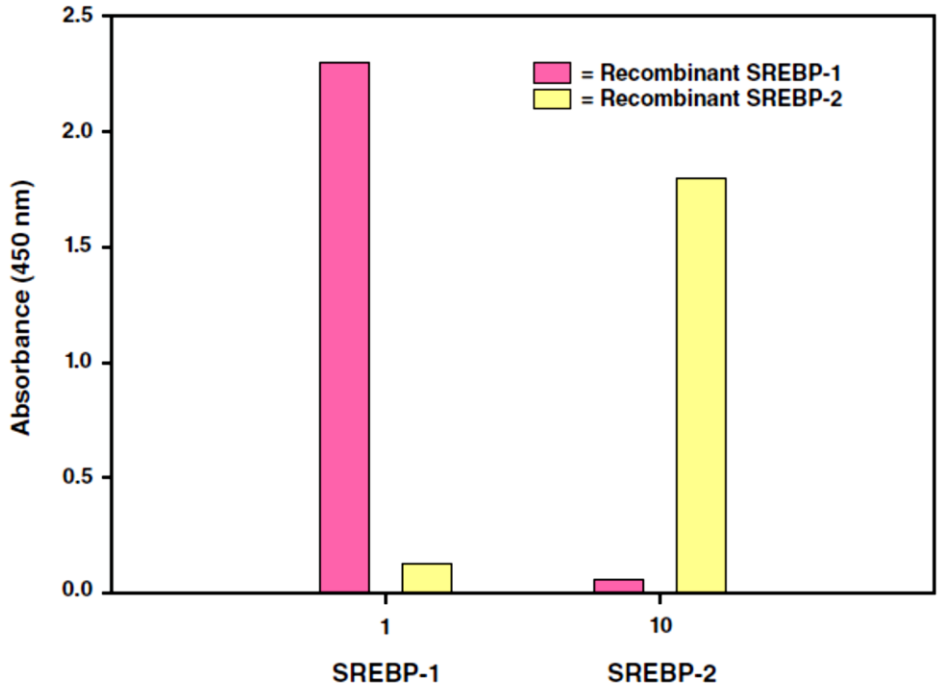

Figure 5. Assay of recombinant SREBP-1 and SREBP-2 in SREBP-1 and SREBP-2 Transcription Factor Assay kits. The results demonstrate little to no cross reactivity of SREBPs in the opposing assay.

Cross Reactivity: (+) SREBP-1a, SREBP-1b, and SREBP-1c

- 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

Resources

Troubleshooting

Problem	Possible Causes	Recommended Solutions
No signal or weak signal in control 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 at 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. Overdeveloping.	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

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6. Ahmed, M.H. and Byrne, C.D. Modulation of sterol regulatory element binding proteins (SREBPs) as potential treatments for non-alcoholic fatty liver disease (NAFLD). *Drug Discovery Today* 12(17/18), 740-747 (2007).

Plate Layout

	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