SREBF2 Cell-Based Translocation Assay Kit

Catalog Number KA1378

96 assays

Version: 02

Intended for research use only

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Background

Lipid homeostasis in vertebrate cells is regulated by a family of basic helix-loop-helix (HLH) transcription factors called sterol regulatory element-binding proteins (SREBPs). SREBPs directly activate the expression of over 30 genes involved in both the synthesis and uptake of cholesterol, fatty acids, triglycerides, and phospholipids.[1,2] Each SREBP, including SREBP-1a, SREBP-1c, and SREBP-2, consists of three domains: an amino-terminal transcription factor domain of ~480 amino acids, a middle hydrophobic region of ~80 amino acids containing two hydrophobic transmembrane segments, and a carboxy-terminal regulatory domain of ~590 amino acids.[3] SREBP-2 regulates cholesterol synthesis by activating the transcription of genes for HMG-CoA reductase and other enzymes of the cholesterol synthetic pathway.[4] It is also involved in activating genes required to generate NADPH, which is consumed at multiple stages in cholesterol biosynthesis. SREBP-2 is ubiquitously expressed.[5] Under basal conditions SREBP is bound to endoplasmic reticulum membranes as a glycosylated precursor protein and SREBP cleavage-activating protein (SCAP) is bound to sterol. Upon depletion of cholesterol, SCAP becomes activated and escorts SREBP to the Golgi where it is proteolytically cleaved by site 1 protease and site 2 protease, S1P and S2P, respectively.[4,6] The active transcription factor consisting of the NH2-terminal domain, designated as nuclear SREBP (nSREBP), is translocated into the nucleus to stimulate transcription of genes involved in the uptake and synthesis of cholesterol. This protein activation characteristic makes it possible to study modulators of SREBP-2 through sub-cellular localization of the protein using immunocytochemical staining with a specific antibody.[7]

Reduction of circulating cholesterol and modulation of lipid biosynthesis have important clinical implications for many diseases including obesity, type 2 diabetes, and atherosclerosis. Thus, identification of SREBP-2 activators/inhibitors can be highly relevant in the search for cures to these diseases.

About This Assay

SREBF2 Cell-Based Translocation Assay Kit provides highly specific SREBP-2 primary antibody together with a DyLight™ (trademarked by Thermo Fisher Scientific.) conjugated secondary antibody in a ready to use format.

Material Supplied

Item	100 Tests Quantity	Storage
Fixative	1 vial/12 ml	Room Temperature
SREBP-2 Primary Antibody	1 vial/100 µl	-20℃
Blocking Solution	1 vial/12 ml	4℃
DyLight™ 549-Conjugated Goat Anti-Rabbit Secondary Antibody	1 vial/100 µl	-20℃
U18666A, 2.5 mM	1 vial/100 µl	-20℃

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

Materials Needed But Not Supplied

- ✓ A 6-, 12-, 24-, or 96-well plate.
- ✓ Raw 264.7 cells (can be obtained from ATCC) or any cells in which translocation of SREBP-2 is active.
- ✓ Immunocytochemical staining buffer, TBS or PBS, pH 7.4.
- ✓ Triton-X 100.
- ✓ A fluorescence microscope equipped with a filter capable of measuring excitation and emission at 550 and 568 nm, respectively.

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.

Performing the Assay

Treatment of the Cells

The following protocol is designed for a 96-well plate. Adjust volumes accordingly for other plate sizes.

1. Seed wells of a 96-well plate with 3 x 10^4 cells/well. Grow cells overnight.
2. The next day, treat cells with or without experimental compounds for 48-72 hours, or for the period of time used in your typical experimental protocol. U18666A, a cholesterol transport inhibitor, is included as a positive control.
3. After the designated incubation period, examine the effect of testing compounds on SREBP-2 activation by the following immunofluorescent staining procedure in section B.

Immunofluorescent staining procedure

NOTE: Perform all steps at room temperature.

The reagent volumes provided below are for use on a 96-well plate. Adjust volumes accordingly for other plate sizes.

1. Remove most of the culture medium from the wells.
2. Wash cells briefly with TBS, pH 7.4.
3. Fix the cells with Cell-Based Assay Fixative Solution for 10 minutes.
4. Wash the cells three times with TBS containing 0.1% Triton-X 100 (TBST) for five minutes each.
5. Incubate the cells with Cell-Based Assay Blocking Solution for 30 minutes.
6. Incubate the cells for one hour with 100 µl of SREBP-2 Cell-Based Assay Primary Antibody diluted 1:100 in TBST.
7. Wash the cells three times with TBST for five minutes each.
8. Incubate the cells in the dark for one hour with 100 µl of DyLight™ 549-Conjugated Goat Anti-Rabbit Secondary Antibody diluted 1:100 in TBST.
9. Wash the cells three times with TBST for five minutes each.
10. Examine the staining under a fluorescent microscope with a filter capable of measuring excitation and

emission at 550 and 568 nm, respectively. Store the plate at 4℃ in the dark for later analysis.

Cell Staining

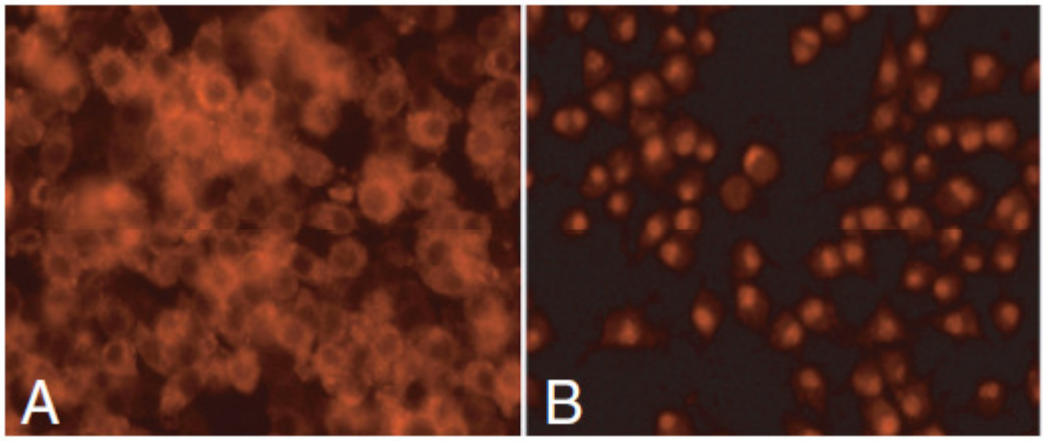

Figure 1: Translocation of SREBP-2 into nuclei by U18666A. Raw 264.7 cells were seeded in a 96-well plate at a density of 3 x 10^4 cells/well and cultured overnight. The next day, cells were treated with DMSO (vehicle) or 24 µM U18666A for 72 hours. *Panel A:* Cells treated with DMSO alone demonstrate cytoplasmic localization of SREBP-2, indicating that most of cells have inactive protein. *Panel B:* U18666A treatment for three days induced SREBP-2 translocation into the nuclei, indicating that blockage of cholesterol transport in these cells activates the protein.

Troubleshooting

Problem	Possible Causes	Recommended Solutions
No signal in any wells	Omission of key reagent	Check that all reagents have been added and were added in the correct order
High signal in all wells	Overgrowth of cells	Make sure to plate cells at low density before starting treatment

References

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2. Lin, J., Yang, R., Tarr, P.T., *et al.* Hyperlipidemic effects of dietary saturated fats mediated through PGC-1β coactivation of SREBP. *Cell* 120, 261-273 (2005).
3. Brown, M.S. and Goldstein, J.L. The SREBP pathway: Regulation of cholesterol metabolism by proteolysis of a membrane-bound transcription factor. *Cell* 89, 331-340 (1997).
4. Sakai, J., Nohturfft, A., Goldstein, J.L., *et al.* Cleavage of sterol regulatory element-binding proteins (SREBPs) at site-1 requires interaction with SREBP cleavage-activating protein. Evidence from *in vivo* competition studies. *J. Biol. Chem.* 273(10), 5785-5793 (1998).

5. Iizuka, K., Bruick, R.K., Liang, G., *et al.* Deficiency of carbohydrate response element-binding protein (ChREBP) reduces lipogenesis as well as glycolysis. *Proc. Natl. Acad. Sci. USA* 101(19), 7281-7286 (2004).
6. Matsuda, M., Korn, B.S., Hammer, R.E., *et al.* SREBP cleavage-activating protein (SCAP) is required for increased lipid synthesis in liver induced by cholesterol deprivation and insulin elevation. *Genes & Development* 15, 1206-1216 (2001).
7. Smith, L.H., Petrie, M.S., Morrow, J.D., *et al.* The sterol response element binding protein regulates cyclooxygenase-2 gene expression in endothelial cells. *J. Lipid Res.* 46, 862-871 (2005).