Trans Genic Inc.

Code No. StDtc-3

Research Reagent Only

OKD Oxidative Stress Detector

Cellular stresses, which are implicated in various diseases, include stresses caused by intra-tissue factors such as low oxygen status and intracellular stresses such as endoplasmic reticulum (ER) stress and oxidative stress. Oxidative stress conditions enhance the production of reactive oxygen species and are associated with various human diseases, including neurodegenerative disorders, inflammation and various cancers.

OKD Oxidative Stress Detector utilizes the expression regulation system of Nrf2 transcriptional factor. Under normal condition, Nrf2 is rapidly degraded by the ubiquitin-proteasome pathway through the association with Keap1. Upon exposure to oxidative stress, reactive cysteine residues in Keap1 are covalently modified, leading to the liberation of Nrf2 from Keap1-mediated degradation. The stabilized Nrf2 is then translocated to the nucleus, and activates the transcription of a wide range of oxidative stress responsive genes. OKD Oxidative Stress Detector is a reporter gene in which luciferase gene is fused with Nrf2 cDNA corresponding to ubiquitination domain. In cells introduced with this detector gene, oxidative stress stabilizes the luciferase fusion protein and induce the luciferase activity.

For detection of oxidative stress in living mouse, Tg type OKD-Luc mouse which is introduced with ERAI ER Stress Detector is suitable.

Material	Plasmid DNA
Quantity	5 µg DNA/vial, 20 µL TE (sterilized)
Storage	- 20℃
	Plasmid is not treated in endotoxin free condition.

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【An example of experimental procedure】

Cultured cells are transfected with OKD Oxidative Stress Detector and stimulated with oxidative stress inducer arsenite.

1) One day prior to transfection, cells are plated in 35 mm culture dish. Cell number should be 50 - 80% confluent after overnight culture (approximately 1 - 3 x 10^5 cells/dish for adherent cells).

2) OKD Oxidative Stress Detector plasmid is transfected. Detail of transfection method follows the manufacturer's instruction of transfection reagent.

3) After 24 - 48 hours, culture medium is exchanged to new one with 10 μM arsenite. Note that the optimum concentration of tunicamycin depends on each cell line.

4) After 6 hours incubation with tunicamycin, cells are harvested and assayed for luciferase activity by Luciferase Assay System (Promega). Please see detailed method in instruction of product.

【References】

Oikawa D., Akai R., Tokuda M., Iwawaki T. Sci. Rep. 2, 229 (2012)

【License statements】

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Production of gene modified animals introduced with this product is prohibited.

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