

Cellular Activation of Signaling ELISA:

CASE™ Kit for NFκB p65 S276

<u>Catalog Number</u>	<u>Contents</u>
FE-007	One CASE Kit for 96 cell-based assays

Description

The CASE™ Kits for NFκB p65 are designed to analyze p65/RelA phosphorylation in cultured human cell lines. These cell-based ELISA kits quantify the amount of activated (phosphorylated) NFκB p65/RelA protein relative to total p65/RelA protein. Phosphorylation of p65/RelA at serine 276 is induced by PKA and MSK1. By determining p65/RelA protein phosphorylation in your experimental model system, you can verify NFκB pathway activation in your cell line and determine the effect of various treatments, inhibitors, or activators on the pathway.

More information on NFκB p65 protein from NCBI:

“NFκB1 or NFκB2 is bound to REL, RELA, or RELB to form the NFκB complex. The p50 (NFκB1)/p65 (RELA) heterodimer is the most abundant form of NFκB. The NFκB complex is inhibited by I-kappa-B proteins, which inactivate NFκB by trapping it in the cytoplasm. Phosphorylation of serine residues on the I-kappa-B proteins by kinases (IKKα or IKKβ) marks them for destruction via the ubiquitination pathway, thereby allowing activation of the NFκB complex. Activated NFκB complex translocates into the nucleus and binds DNA...”

Reference: www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=nucleotide&val=46430498

Materials Included

Please check the kit components immediately after you receive this package. SuperArray is not responsible for any missing items not reported within two (2) business days upon receipt.

Shelf Life: All reagents are stable at the recommended temperature for 6 months after receipt of the kit.

Enough reagents are provided for 96 assays.

Box 1: Assay and Detection Reagents (Store at 4°C)

Components
10X Antigen Retrieval Buffer ²
10X Washing Buffer ²
Antibody Dilution Buffer
Blocking Buffer, dry powder ³
Cell Staining Solution
Developing Solution
Secondary Antibody ¹ (Dilute 1:16 before use.)
Stop Solution
Washing Buffer Reservoir (4)

Box 2: Primary Antibodies¹ (Store at -20°C)

Components	Dilution Factor
Anti-Phospho-Protein Specific Antibody	1:100
Anti-Pan-Protein Specific Antibody	1:100

¹Each antibody stock must be freshly diluted by the appropriate factor into Antibody Dilution Buffer on the same day of the experiment. See the CASE™ Kit User Manual for more details.

²10X Washing Buffer and 10X Antigen Retrieval Buffer must be diluted to 1X with dH₂O before use.

³Just before use, dissolve powder in 30 ml 1X Washing Buffer by mixing well. Once dissolved, Blocking Buffer may be stored at 4 °C for up to two (2) weeks.

Shipping and Storage Conditions

Box 1 is shipped at either ambient or a refrigerated temperature and should be stored at 4°C.
Box 2 is shipped on dry ice or cold packs and should be stored at -20°C.

Related Products

Inhibitors for Functional Assays: Inhibitors of pathway activation that serve as a control for specificity.

For follow-up at the expression level:

RT² Profiler™ PCR Arrays and Oligo GEArray® Microarrays
(See www.superarray.com/ArrayList.php for complete listings)

RT² Real-Time™ PCR Primer Sets – available for every human, mouse, and rat gene
(See www.superarray.com/QRTsearch.php for our primer set search engine)

For knock down of gene expression:

SureSilencing™ shRNA Plasmids – available for every human, mouse, and rat gene
(See www.superarray.com/RNAisearch.php for complete listings)