Product Datasheet

IkBa Mutant Adenovirus Assay Kit
NBP2-29443

Unit Size: 1 Kit

Store at -80C. Avoid freeze-thaw cycles.

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Publications: 2

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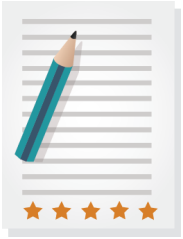

NBP2-29443

IkBa Mutant Adenovirus Assay Kit

Product Information	
Unit Size	1 Kit
Concentration	Concentration is not relevant for this product. Please see the protocols for proper use of this product.
Storage	Store at -80C. Avoid freeze-thaw cycles.

Product Description	
Description	IkBa dominant mutant (IkBaM) is a mutated form of IkBa with a serine-to alanine mutations at residues 32 and 36 (8). Overexpression of IkBaM presumably prevents phosphorylation of native IkBa and there by inhibiting translocation of NF-kB complex to the nucleus and thereby preventing NF-kB activation. In most cell lines, the inhibition of NF-kB activation affects cell growth. The IkBaM was tested on proliferation of HeLa cells in tissue culture plates.
Reactivity Notes	Human.
Kit Components	Control virus at a concentration of 1 x 10^11 (virus particle/ml) Volume: 25 ul Amount of virus: 2.5 x 10^9, I?BaM virus at a concentration of 1 x 10^11 (virus particle/ml) Volume: 25 ul Amount of virus: 2.5 x 10^9
Notes	Human IkBa deletion mutant adenovirus. Addition of this virus to cell culture inhibits NF-kB activation. The virus is replication incompetent in non-permissive host cells due to the deletion of genes required from viral replication.

Publications

Kusne Y. The Dissection of Signaling Cascades in Neural Stem Cell Proliferation & GBM Promotion Thesis. 2014-04-01

Details:
IkBaM adenovirus used for induction of various genes in U251/EGFR cells treated with 100 ng/mL EGF for 6 h or 10 ng/mL TNF alpha for 1 h (Figure 3.S13 E and F)

Wang Z, Castresana MR, Detmer K et al. An IkappaB-alpha mutant inhibits cytokine gene expression and proliferation in human vascular smooth muscle cells. J Surg Res. 2002-02-01 [PMID: 11796019] (WB, Human)

Details:
Construction of the IMG-2500 IkBa deletion mutant adenovirus (IkBaM) (Materials and Methods); Primary human vascular smooth muscle cells (VSMC) growing in culture infected with IkBaM and analyzed for IkBaM expression by WB using an IKBa antibody (Fig 1);

Procedures

Product Handling Protocol (NBP2-29443)
EXPERIMENT:
Test of IkBaM adenovirus on cell growth in HeLa cells.

REAGENTS

Cells: HeLa cells.

Virus: IkBaM Adenovirus or control adenovirus in PBS containing 3% Sucrose.

MTT solution: 3-(4, 5 dimethylthiazol-2-yl)-2, 5 -dimethyltetrazoloium bromide) (MTT) A 0.5mg/ml solution was prepared in PBS.

SDS/DMF reagent: (20% SDS in 50% DMF): A 40 % solution of SDS was first prepared in water. This solution was diluted by half with N,N'Dimethylformamide.

PROCEDURE

This protocol is written for HeLa cells. However, this can be adapated to the cell line of your choice.

Preparation of cells:

1. Prepare HeLa cells grown to confluence in a 100 mm culture dish.

2. Trypsinize, wash the cells and seed at 10,000 cells per well in a 96-well plate.

3. Twenty-four hours later replace the medium with DMEM containing 2% fetal bovine serum.

4. Infect cells with the control or test virus at various dilutions.

Recommended concentration is 1:100, 1:1,000 and 1:10,000. Dilute the viruses in DMEM containing 2% fetal bovine serum (1:100 dilution would result in approximately 100 viral particles per cell).

MTTAssay:

1. After 24 hrs, remove the medium and wash the cells with PBS.

2. Add twenty-five micro liters of MTT solution per well. After 40 minutes of incubation at room temperature, add 100 ul of SDS reagent was added per well.

3. Read the plate in a micro-plate reader using the filter 595 nm filter.

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