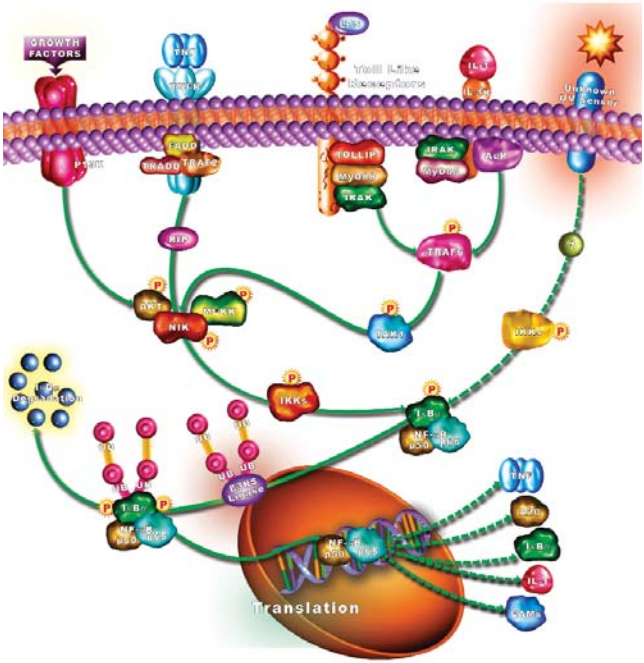
Human IκBα Mutant Virus

For Inhibition of NF-κB activity

Catalog No. IMG-2500

NF-κB Family Pathway



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Human IκBα Mutant Adenovirus

For inhibition of NF–κB activity

(version 08/03)

Catalog No.: IMG-2500

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A. Kit Components and Storage:

The components included in this kit need to be aliquoted stored at -70°C. Avoid multiple freeze-thaw.

Item	Conc. (pt/ml)	Volume	Amount of virus
IκBαM virus	1 x 10^{11}	25 µl	2.5 x 10^{9}
Control virus	1x 1011	25 µl	2.5 x 10^{9}

pt= virus particle.

B. Background:

NF-κB is controlled by a family of inhibitory proteins called, IκBs (1). IκB proteins are phosphorylated by IκB kinase complex consisting of at least three proteins, IKK1/α, IKK2/β, and IKK3/γ (2-5). External stimuli such as tumor necrosis factor or other cytokines initiates a signal transduction cascade that leads to the activation of IκB-kinase complex that specifically phosphorylates IκBα on Serine-32 and Serine-36. Phosphorylation of these sites leads to ubiquitination of IκBα and subsequent degradation by the 26 S proteasome. Degradation of IκBα results in unmasking of the nuclear localization signal of NF-κB dimers, which subsequently translocates to the nucleus and activates target genes (6,7). Thus analysis of the phosphorylation of IκBα has a correlation to the activity of the IKK complex as well as the nuclear localization and activation of NF-κB.

C. Overview:

IκBα dominant mutant (IκBαM) is a mutated form of IκBα with a serine-to alanine mutations at residues 32 and 36 (8). Overexpression of IκBαM presumably prevents phosphorylation of native IκBα and there by inhibiting translocation of NF-κB complex to the nucleus and thereby preventing NF-κB activation. In most cell lines, the inhibition of NF-κB activation affects cell growth. The IκBαM was tested on proliferation of HeLa cells in tissue culture plates.

D. References:

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3. Regnier, C.H., Ho, Y.S., Gao, X., Goeddel, D.V., Cao, Z., and Rothe, M. *Cell* 90, 373 (1997).
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6. Ghosh, S., May, M.J., and Kopp, E.B. Annu Rev. Immunol. 16: 225-260 (1998).
7. Baldwin, A.S. *Annu. Rev. Immunol.* 14: 694-681 (1996).
8. Feig, B.W., Lu X, Hunt K.L., San Q, Yu D, Pollock R, and Chiao P. *Surgery* 126: 399-405 (1999).

E. EXPERIMENT:

Test of IκBαM adenovirus on cell growth in HeLa cells.

REAGENTS

Cells: HeLa cells.

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

MTT solution: 3-(4, 5 dimethylthiazol-2-yl)-2, 5 -dimethyltetrazoloium bromide) (MTT) (Sigma cat # M2128). 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 (Sigma D-8654).

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).

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MTT Assay:

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 µl of SDS reagent was added per well.
3. Read the plate in a micro-plate reader using the filter 595 nm filter.

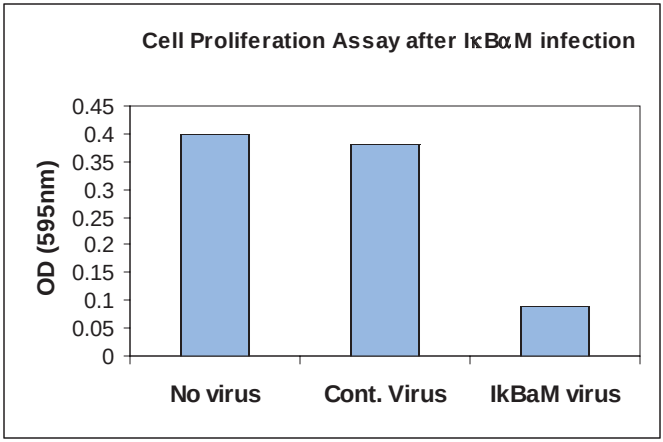

Figure 1. Effect of IκBα dominant-negative mutant expression on HeLa cell proliferation.

F. Safety Considerations:

Recombinant Ad 5 vectors produced using Imgenex vectors are replication impaired. However, all the materials should be handled in a Biosafety Level: NIH BSL 2 laboratory.

For more information on biosafety levels, please refer to the following CDC publication: Biosafety in Microbiological and Biomedical Laboratories, 4th Edition, May 1999; this publication is also available at http://bmbl.od.nih.gov.

SECTION I - HEALTH HAZARD

Pathogenicity: Wild type Adenovirus symptoms may include fever, rhinitis, pharyngitis, cough and conjunctivitis. The risk from infection by defective recombinant adenoviral vectors depends both on the dose of virus and on the nature of the transgene. Adenovirus does not integrate into the host cell genome but can produce a strong immune response.

Host Range: Humans are the natural reservoir for wild type Adenovirus 5. Recombinant Adenovirus vectors infect a variety of mammalian cell types.

Mode of Transmission: Wild type virus is spread directly by oral contact and droplet spread; indirectly by handkerchiefs, eating utensils and other articles freshly soiled with respiratory discharge of an infected person. In the laboratory, care must be taken to avoid spread of infectious material by aerosol, direct contact or accidental injection.

Incubation Period: From 1-10 days.

Disinfectants: 1% sodium hypochlorite, 2% glutaraldehyde. Recommend fresh solution of 10% bleach for 30 minutes.

Physical Inactivation: Sensitive to heat; 1 hour at 56°C is used to inactivate virus.

Survival Outside of Host: Adenovirus has been reported to survive 3-8 weeks on environmental surfaces at room temperature

First Aid/Treatment: For splashes to the eye of material containing virus, rinse eye at eyewash for 15 minutes then report to nearest hospital emergency room for evaluation. A serum sample should be taken as soon as possible. In the case of accidental injection of material containing virus, wash area well with soap and water then contact Institutional office of Occupational Health for advice, evaluation and serum sample.

SECTION V - LABORATORY HAZARDS

Laboratory-acquired infections: Rare cases reported in laboratories working with clinical specimens.

Sources/Specimens: Respiratory secretions. Theoretical risk from exposure to laboratory cultures of wild type virus or recombinant virus.

Primary Hazards: Ingestion, droplet exposure of the mucous membranes, direct injection.

Special Hazards: Contact with feces or urine from infected animals for 72 hours post infection.

SECTION VI - RECOMMENDED PRECAUTIONS

Containment Requirements: Biosafety level 2 plus Institutional Adenoviral special practices and BSL 2 containment facilities for all activities involving the virus, recombinant virus vectors, and potentially infectious body fluids or tissues.

Protective Clothing: Laboratory coat, gloves, goggles.

SECTION VII - HANDLING INFORMATION

Spills: Allow aerosols to settle for 15 minutes; wear protective clothing and gently cover the spill with adsorbent paper towel and apply freshly prepared 10% sodium hypochlorite starting at the perimeter and working towards the center; allow at least **3**0 minutes contact time before clean up.

Disposal: Decontaminate all wastes before disposal; steam sterilization, incineration, chemical disinfection (10% bleach).

Storage: In sealed containers that are appropriately labeled and in approved locations for BSL 2 materials at –20 or -80°C.

Transport: Material must be sealed in primary and secondary containers, appropriately labeled.

If you require additional assistance, please contact Imgenex Technical Service:

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Related Products:

Catalog No.	Antigen	Reactivity	Application*
IMG-161	A20	Human	WB, IP
IMG-284	Act1	Human	WB
IMG-187	Phospho-AKT1	Human	WB
IMG-127	IκBα	Human, mouse	WB
IMG-156	Phospho-IκBα	Human	WB
IMG-136	IKK1/α	Human, mouse, monkey	WB, IP
IMG-129	IKK2/β	Human	WB
IMG-159	IKK2/β	Human	WB, IP
IMG-287	IKKγ	Human	WB
IMG-324	IKKγ	Human, mouse	WB
IMG-139	IKKδ/TBK1	Human	WB
IMG-270	IKKε/i	Human	WB
IMG-309	IKKε/i	Human	WB
IMG-179	IRAK	Human	WB
IMG-180	IRAK-2	Human	WB
IMG-390	κBRas-1	Human	WB
IMG-178	MyD88	Human	WB
IMG-150	NF-κB (p65)	Human	WB
IMG-243	TAB1	Human	WB
IMG-244	TAB2	Human	WB

* WB= western blotting; IC= Immuno cytochemistry; IP= Immunoprecipitation

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