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Adenoviral human IκBα siRNA ReadyVirus™

- Catalog No.:** **IMG-1205VS** (1 x 10¹² viral particles/ml, 25 μl)
IMG-1205VL (1 x 10¹² viral particles/ml, 100 μl)
- Lot no.** 040504
- Contents:** Adenoviral human IκBα siRNA Recombinant Virus
Anti-IκBα antibody (IMG-127), 100 μg (0.5mg/ml in 200ul PBS with 0.02% sodium azide; Isotype: Mouse IgG). Sodium azide is highly toxic.
- Storage:** Store the viral particles at -80°C. For multiple uses, we suggest aliquoting the stock solution prior to freezing. Store the antibody at 4°C for up to six months; store at -20°C for longterm storage.
- Caution:** A safety protocol for handling the viral particles is attached. However, we strongly recommend that you follow your Institutional guidelines.

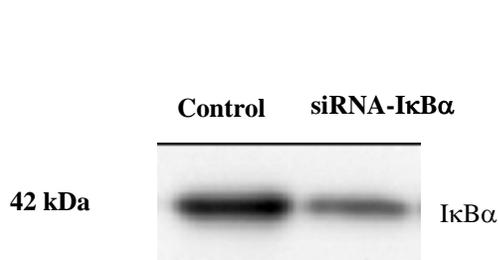
Background

NF-κB (nuclear factor κB) is sequestered in the cytoplasm by IκB family of inhibitory proteins that mask the nuclear localization signal of NF-κB thereby preventing translocation of NF-κB to the nucleus. External stimuli such as tumor necrosis factor or other cytokines results in phosphorylation and degradation of IκB releasing NF-κB dimers. NF-κB dimer subsequently translocates to the nucleus and activates target genes. Synthesis of IκBα is autoregulated. IκB proteins are phosphorylated by IκB kinase complex consisting of at least three proteins, IKK1/α, IKK2/β, and IKK3/γ. In vitro, IKK1/α and IKK2/β can form homo- and heterodimers that can phosphorylate IκBs at the regulatory serine residues directly.

Protocol for virus infection (This protocol is routinely used for quality control testing of the adenoviral IκBα siRNA. Users should optimize the protocol for their cell line and model system)

1. H1299 (a human colon carcinoma cell line) cells were plated on a 6 well plate a day before infection. (5 x 10⁵ cells /well).
2. After 24h post-plating, viruses were diluted in 1 ml of media (DMEM, 2% FBS, 1% Penn/Strep), per well with a concentration of 1000 particles of virus per cell. (We suggest users titrate the virus from 100 to 1000 particles/cell, leaving one well uninfected as a negative control).
3. Old media was aspirated from the plate and 1 ml of media containing virus was added to each well.
4. Plates were incubated at 37°C in CO₂ incubator for 48 h.
5. After 48 h, cells were washed in PBS and 200 ul of lyses buffer was added.
6. Protein quantity was estimated by BioRad protein assay.
7. Ten microgram protein was used for western blot.

Figure 1: Western blot analysis of I κ B α suppression



H1299, colon carcinoma cells were infected with I κ B α siRNA or left uninfected (control). Whole-cell extracts were prepared, equal amounts of protein were separated by SDS page and then analyzed by western blot using the I κ B α antibody (Cat No. IMG-127).

SAFE USE OF ADENOVIRUS IN THE LABORATORY:

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://bmbi.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 30 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 -80oC.

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