

Mouse lung tissues were lysed with the buffer [1% SDS, 10 mmol/L Tris-Cl (pH 7.6), 20 µg/µL aprotinin, 20 µg/µL leupeptin, and 1 mmol/L 4-(2-aminoethyl)benzenesulfonyl fluoride]. The protein concentrations were determined using the Bicinchoninic Acid Protein Assay kit (Pierce, Rockford, IL). Twenty micrograms of protein were separated on SDS-PAGE gels and transferred to polyvinylidene difluoride membranes. After blocking, the membranes were incubated with the appropriate primary antibody at 4°C overnight. After washing with TBST, the membranes were incubated with HRP-conjugated secondary antibodies at room temperature for 1 hour. Proteins were detected with the enhanced chemiluminescence kit (Amersham Pharmacia Biotechnology, Inc., Piscataway, NJ).