

Bleomycin induced pulmonary fibrosis

1. Prepare bleomycin stock: dissolve 15 units bleomycin in 6 ml sterile saline to make stock solution 2.5 U/ml.
2. Age and sex matched mice are anesthetized with isofluorane and the trachea is exposed. Bleomycin (1.1 U/kg body weight) in 60 μ l volume or saline are injected transtracheally by direct cut down.
3. Animals will be sacrificed 20 days and 60 days after bleomycin injection and lungs will be collected for hydroxyproline assay.
4. Perfuse lungs with PBS, weight and freeze in liquid nitrogen and store at -80°C .
5. Hydroxyproline assay:
 - a. Thaw lungs in 1 ml water, homogenize in Fisher glass tubes (#14961).
 - b. Add 125 μ l 50% TCA (trichloroacetic acid T6399-Sigma) to the homogenate and incubate on ice for 20 minutes.
 - c. Spin samples at 1000 rpm, 5 min. 4°C . Supernatant is discarded and 1 ml 12N HCL is added to the pellet in glass tube. Bake at 110°C for 24 h (in a glass beaker).
 - d. Reconstitute dried pellet with 2 ml dH_2O . Make up 6 hydroxyproline standards (Sigma-H6002) starting from 0.25 mg/ml.
 - e. In an 1.5 ml eppendorf tube containing 500 μ l chloramine T (1.4% chloramine T in 0.5 M Na Acetate and 10% isopropanol)
 - (i). Add 200 μ l sample and incubate 20 minutes at room temperature.
 - (ii). Add 500 μ l of Ehrlich's/pDMAB (1 M p-DMBA (p-dimethylaminobenzaldehyde) in 70% isopropanol and 30 % perchloric acid) incubate at 65°C for 15 min.
 - (iii). Transfer 100 μ l of final reaction solution to 96-well plate, triplicate measurement for each sample, read at 550 nm.