



## Insulin Human AF Dilution Instructions

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- A) Suspend the insulin product in WFI or purified water.
- B) Dissolve the insulin product by adjusting the pH to 3 with diluted HCl (0.1 or 0.01M, depending on the desired volume of growth medium). The insulin solution should not be held at pH 3 for more than one hour.
- C) Readjust pH to 7.4 with buffer or base. The solution becomes cloudy when passing through the isoelectric point of the insulin product, but becomes clear again as the pH of the solution reaches 7.
- D) The solution should be sterile filtered using a low protein binding filter with a pore size of 0.2  $\mu\text{m}$ . Aeration and foaming should be avoided during solubilization and filtration, as this can lead to protein denaturation.
- E) The recommended concentration of Insulin Human in cell culture media is 1 - 10 mg/L, depending on cell type and specific application.

Solubility: Soluble at 10 mg/mL in pH 2 - 3

Activity:  $\geq 27.5$  IU/mg (dry basis)