## **Diagram: tPA Downstream Process Flow Chart**

Bioreactor: End of Run – conditioned medium

Centrifugation to pellet cells



Supplementation with protease inhibitors (aprotinin, leupeptin, PMSF) & Tween 80 (0.1%)



Concentration by tangential flow filtration; final volume ~20 ml + 10 ml rinse



Acidification: adjustment of pH to 5.0 (add acetic acid dropwise; transfer to Oak Ridge tubes; incubate on ice 30 min to ON; centrifugation at 10000xg, 20 min)



Further concentration via Centricon 15 (30k MW cutoff; 20+ minutes, 4000xg)



Cation Exchange Chromatography on AKTA pure system.



Analysis of fractions by ELISA.



SDS-PAGE of EOR concentrate and tPA-containing fractions.