PREPARATION OF EXTRACTS FROM HUMAN PLACENTA

Materials:
Buffers A, B, and D are made up according to formulas given under Preparation of Nuclear Extracts.

WARNING: The following procedure involves handling fresh human tissue and there is a serious biohazard risk. Donors of placentas are not tested for infectious human pathogens, including hepatitis and AIDS virus. You must therefore assume that the placenta you obtain could carry these pathogens. Take all precautions to prevent any placental blood or tissue from coming into contact with your skin. Wear latex gloves and full length lab coat. Be exceedingly careful during those steps where sharp implements are used (scissors for mincing tissue, etc.). Lastly, all leftover tissue must be wrapped in labtop soaker and delivered to animal facilities for incineration and all plastic centrifuge bottles, glassware, etc., used during the procedure should be rinsed, washed (while wearing gloves), and autoclaved.

Procedure:
1. Obtain one fresh full-term human placenta within 30 minutes of delivery (Delivery room-Evanston Hospital). Drain excess blood, place placenta in red plastic tissue bag (provided at Delivery room), and then pack in ice in igloo cooler for transport back to lab.

2. Section placental cotyledons with scissors (cotyledons are bundles of villi on the uterine side of placenta-if you are unfamiliar with placental anatomy, it is best to first go to Department of Pathology, Evanston Hospital where someone can show you a placenta and point out the cotyledons). Take only pink, spongy tissue, and avoid hard, small yellow nodules (if any). These yellow nodules are placental infarctions and contain dead tissue. Lastly, section into cotyledons until you begin to see white stringy cords. This is connective tissue and you don't want it.

3. As you take pieces of placenta place them in a beaker on ice. All subsequent steps are done at 4°C. At intervals, use scissors to finely mince tissue in beaker and place it in a large plastic centrifuge bottle on ice which contains ice-cold 1 X PBS (2 tissue vols.). After all tissue is minced, swirl in bottle in 1 X PBS for 1 min. to wash out blood cells. Spin in GSA rotor in Sorvall at 2K rpm for 1 minute. Carefully pour off wash, and repeat with another 1 X PBS wash (1 min.) and GSA spin.

4. At this point you can freeze away tissue you don't need by placing in 50 ml culture tubes, putting in liquid N_2 for 2 minutes, and storing at -80°C. For most purposes 40-50 mls of fresh tissue for processing is more than sufficient.

5. Place fresh tissue (or thawed tissue that had been frozen if you want whole cell extracts) in Waring Blender in cold room with an equal volume Buffer A. Homogenize for 1.5-2 minutes. Pour into Corex tubes and pellet large debris by centrifuging in HB-4 rotor at 2K rpm, 1 minute. Take supernatant. At this point cells in supernatant are usually broken but I like to use an additional 10 strokes with a Dounce homogenizer (B pestle) just to be sure. Examine under microscope for complete lysis. At this point you can isolate nuclei by pelleting at 2K rpm, 10 minutes. (For nuclear extracts follow the instructions given under Preparation of Nuclear Extracts).

6. To cytoplasmic supernatant add 0.11 vols. Buffer B. Centrifuge at 100,000g for 30 minutes. Dialyze the high speed supernatant against 20 vols. of Buffer D for five to eight hours. Freeze in aliquots in liquid N_2 and store at -80°C.