Native (non-denaturing) PAGE and western blot

(with Novex Tris-Glycine gels)

Note #1 All buffers, gels and samples used in native PAGE and western blot should be pre-equilibrated at 4°C. Electrophoresis and blotting should be performed at 4°C.

(1) Mix protein sample in Tris-glycine native sample buffer (2x). Do not heat samples for native electrophoresis.

(2) Prepare 1x Tris-Glycine native running buffer by adding 100 ml Tris-Glycine native running buffer to 900 ml of mQ water. Fill the electrophoresis chamber with 1x Tris-Glycine native running buffer.

(3) Load the protein sample onto a 4-20%Tris-Glycine gel. Run electrophoresis starting at a constant current of 10 mA and after the samples have entered the gel, usually within 1 hr, the current is increased to 15 mA. The duration of electrophoresis will depend on the acrylamide concentration and the degree of separation desired. I usually run the gels for 5-6 hr. Gels are stained with Coomassie blue R250 at room temperature or prepared in the cold for electrotransfer to polyvinylidene difluoride (PVDF) membranes.

(4) For blotting native gels, use 1x Tris-Glycine native transfer buffer. Methanol and SDS are omitted from the Tris-glycine transfer buffer. Electrotransfer and all immunoblotting steps through the final membrane wash are performed at 4 ° to maintain the conformation of the protein. In both denaturing and nondenaturing electrotransfer procedures, the PVDF membrane must be prewetted with methanol in order to enable macromolecules in the aqueous phase to bind to the hydrophobic surface of the membrane. For nondenaturing applications, the membrane is then rinsed repeatedly with Tris-glycine buffer to remove all he methanol before use. Perform transfer at 20 V for 2 hr using the Xcell II Blot Module. The expected current is 100 mA.