Section 4
Native PAGE

4.1 Introduction

Ready Gel Tris-HCl gels are made without SDS, allowing separation of protein in their native conformation. The nonreducing and nondenaturing environment of native PAGE allows the detection of biological activity and can improve antibody detection. Native PAGE can also be used to resolve multiple protein bands where molecular mass separation by SDS-PAGE would reveal only one.

Native PAGE uses the same discontinuous chloride and glycine ion fronts as SDS-PAGE to form moving boundaries that stack and then separate polypeptides by charge to mass ratio. Proteins are prepared in a nonreducing nondenaturing sample buffer, which maintains the proteins' secondary structure and native charge density. Native PAGE is not suitable for accurate molecular weight determination due to the variability of charge to mass ratio among different proteins.

4.2 Ready Gel Tris-HCl Gel Composition

Gel buffer	0.375 M Tris-HCl, pH 8.8
Cross-linker	2.6% C
Stacking gel	**4% T, 2.6%** C
Storage buffer	0.375 M Tris-HCl, pH 8.8
Shelf life	~12 weeks

4.3 Ready Gel Tris-HCl Gel Selection

Native PAGE separates by charge to mass ratio, making individual protein migration protein dependent. Optimal Tris-HCl gel percentages will have to be determined experimentally.

4.4 Native PAGE Buffers

Running Buffer

Working Concentration
25 mM Tris
192 mM glycine

10x Stock

Tris base	15.0 g
Glycine	72.0 g

to 500 ml with deionized water

Note: running buffer should be ~ pH 8.3. Do not adjust the pH.

Sample Buffer

Working Concentration
62.5 mM Tris-HCl, pH 6.8
25% glycerol
0.01% Bromophenol Blue

0.5 M Tris-HCl, pH 6.8	1.0 ml
Glycerol	2.0 ml
Bromophenol Blue	1.0 ml
Deionized water	4.9 ml
	8.0 ml

4.5 Sample Preparation

Determine the desired protein concentration and load volume of your sample based on the detection method used. (See section 10.1 for approximate stain sensitivities). Sample preparation for native PAGE applications requires special consideration. In the absence of SDS, the net charge of a polypeptide will be determined by the pH of the sample buffer. Only polypeptides with a net negative charge will migrate into a native PAGE Tris-HCl gel. Most polypeptides have an acidic or slightly basic pI (~3–8). These proteins can be separated using a standard protocol by diluting 1 part sample with 2 parts native sample buffer (see section 4.4; DO NOT HEAT SAMPLES).

Strongly basic peptides (pI >9) will have a net positive charge in a native PAGE Tris-HCl gel. In order for polypeptides with a net positive charge to migrate into a native PAGE Tris-HCl gel, the polarity of the electrodes must be changed by reversing the color-coded jacks when connecting to the power supply.

4.6 Running Conditions

Power conditions	200 V constant	
	Starting current:	50 mA/gel
	Final current:	30 mA/gel
Run time	35 min	