VEGF Western on Tricine Gel

Materials req'd: Tricine gel

Tricine-SDS sample buffer (450mM Tris-Cl, pH 8.45, 12% Glycerol, 4% SDS, 0.0025% Phenol red)

Tricine-SDS Running buffer (100mM Tris base, pH 8.3, 100mM Tricine, 0.1% SDS)

Transfer buffer (for 1L, 800ml dH2O, 3.03g Tris-Base, 14.25g Glycine, 200ml MeOH)

Blocking buffer: 5% BSA in TBST

Wash buffer: TBST (for 3.5L, 3.2165L dH2O, 175ml of 1M Tris-Cl pH 7.4, 105 ml of 5M NaCl, 3.5 ml of Tween-20)

1. Prepare 20% Tricine gel (prepare the night before)
2. VEGF samples + sample buffer containing β-mercaptoethanol (5% in sample buffer), Boil at 95°C, 5 min.
3. Run gel, start with 100V and then increase to 150V after sample ran through stacking gel
4. Transfer 450 mA for 2 hrs or 250 mA O/N.
5. Blocking, RT 1hr or 4°C O/N
6. Primary antibody, #463 (1:1000 dilution), 4°C, O/N
7. Wash with TBST, 3X, 15 min each.
8. Secondary antibody (HRP-anti rabbit), RT 1hr
9. Wash with TBST, 4X, 15 min each.
10. Rinse blot with dH2O
11. Incubate with ECL soln., RT, 2min.
12. Develop

** Recipe for 20% Tricine gel

	Separating	Stacking
37% Acrylamide	16.2 ml	1.3 ml
3M Tris-Cl, pH 8.45	10 ml	3.1 ml
10% SDS	0.3 ml	0.1 ml
Glycerol	3.17 ml	
H2O	2.0 ml	8.1 ml
10% APS	100µl	50µl
TEMED	10µl	5µl