

Tricine Gel after VEGF Digestion

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
Blocking buffer: 2% BSA in TBST
Wash buffer: TBST
HRP-Avidin D (Vector, A-2004)

1. Prepare 20% Tricine gel
2. Reduce digested VEGF samples
Sample buffer containing b-MeOH
Boil at 95°C, 5 min.
3. Run gel
4. Transfer 450 mA for 1.5 hrs or 250 mA O/N.
5. Blocking, RT 1hr or 4°C O/N
6. Wash with TBST, 4X, 15 min each.
7. Incubate with HRP-Avidin D, RT, 45 min – 1 hr.
 1. Dilute HRP-Avidin D in TBST, 1:2000 dilution
8. Wash with TBST, 4X, 15 min each.
9. Incubate with ECL soln., RT, 2min.
10. 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	100ml	50ml
TEMED	10ml	5ml