Western Blot Protocol

- Run the appropriate percentage of SDS-PAGE. Load samples in desired amounts (for Arabidopsis samples, load 6 to 8 ul per lane or 40ug to 80ug per lane). Transfer to nitrocellulose membrane at 100 V for 1h, or 20 V overnight.

- Place the membrane in appropriate volume of Blocking solution (5% milk/1xTBST) and shake at RT for 1 h.

- Add the primary antibody in Blocking solution at appropriate concentration (1:500 to 1:2,000) and incubate at RT for 2 h with shaking.

- Wash 3 times with 15 ml 1xTBST, 10 min per wash at RT with shaking.

- Incubate the membrane in the appropriate secondary antibody (anti-mouse or rabbit) at 1:4,000 in Blocking solution at RT for 1 h with shaking.

- Wash 3 times with 15 ml 1xTBST, 10 min per wash at RT with shaking.

- Develop in ECL and expose for data.

Abiocode, Inc., 29397 Agoura Rd., Ste 106, Agoura Hills, CA 91301

Buffers:

10x Tris-glycine

	Per 1000 ml	Per 2000 ml
Tris-base	30.3 g	60.6 g
Glycine	144 g	288 g
add ddH2O to final volume of:	1000 ml	2000 ml

1x SDS *Running Buffer*

	Per 1000 ml
10x Tris-glycine Buffer	100 ml
10% SDS (w/v)	10 ml
ddH2O	890 ml

1x Tris-glycine *Transfer Buffer*

	Per 1000 ml
10x Tris-glycine Buffer	100 ml
Methanol	200 ml
ddH2O	700 ml

10x TBST

	Per 1000 ml
1.0M Tris-HCl (pH 8.0)	100 ml
NaCl	87.7 g
50% Tween-20	10 ml
Add ddH2O to final volume of:	1000 ml

SDS loading buffer

Stock	Final	2x (10 ml)	4x (10 ml)
1 M Tris, pH 6.8	(0.1M)	1 ml	2 ml
1 M DTT	(0.2M)	2 ml	4 ml
10% SDS	(4%)	4 ml	0.8 g (powder)
BPB	(0.2%)	20 mg	40 mg
100% Glycerol	(20%)	2 ml	4 ml
dH2O		1 ml	0