KDM5D antibody

Cat#: orb387807 (Protocol)

Western Blot Protocol

Running protein gel:
Run SDS-PAGE gel in 1xSDS running buffer at constant current: Stacking gel, 15 mA/gel
Separating gel, 20 mA/gel

Note: for large proteins with MW over 200kD, we recommend to run 6% PAGE gel using Acrylamide/Bis mixture ratio at 37.5:1. Run the 80 kD marker close to the bottom of the gel.

Gel transfer:
Transfer onto NC membrane at 100V for 1h in 1xTransfer Buffer at 40C

Blot
-Place NC membrane in appropriate volume of 5% non-fat milk/1xTBST (Blocking Soln) for 1 hr
-Add Primary Antibody into Blocking Soln at appropriate concentration (1:500 to 1:2,000)
-RT (23oC on the shaker), 2hr shaking (for most polyclonal antibodies) or 40C overnight (for most monoclonal antibodies)
-Wash 3 times with 1xTBST, RT with shaking, 10 min/wash
- Incubate NC membrane in appropriate Secondary Antibody (anti-mouse or rabbit) at 1:5,000 in 5% non-fat milk/1xTBST, RT 1hr with shaking
-Wash 3 times with 1xTBST, RT, 10 min/wash
-After ~5 sec in contact with ECL, expose for appropriate times

Western 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 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 sample 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