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H00002817-A01 Protocol

Protocol specific for Glypican 1/ GPC1 Antibody (H00002817-A01)

Protocol specific for Glypican 1/ GPC1 Antibody (H00002817-A01): https://www.novusbio.com/products/glypican-1-antibody_h00002817-a01
ELISA Protocol

1. Coat antigen (200 ng/well) onto the wells in a 96 well mictrotiter plate.
2. Block unbound sites with 5% skim milk in PBS.
3. Apply hybridoma culture supernatant/ ascites/ purified Ig as primary antibody. Incubate the plate at room temperature for two hours.
4. Wash 4 times with PBST.
5. Apply HRP conjugated secondary antibody, and incubate the plate at room temperature for one hour.
6. Wash 8 times with PBST.
7. Apply 0.1 ml OPD in citric acid buffer, and incubate at room temperature for 20minutes. Read the plate in ELISA reader at 450 nm.
- Between each step, plates were adequately washed using PBST.
- Secondary antibody dilution, 1:1000.
Primary Antibody/Dilution:
- Poly sera @ 1500X
- Cultured Supernatant @ 1X
- Ascites @ 1000X
- Purified Ig @ 1 ug/ml
Diluents:
- 5% skim milk in PBST
Material:
- PBST, 0.2% Tween 20
- Citric acid buffer, pH 5.0
- OPD: Sigma, P-1526

Western Blot Protocol

1. Antigens were denatured and loaded onto polyacryamide gel (200 ng/ lane). Run the gel at 150V for 80minutes when samples enter separating gel.
2. Transfer antigens onto PVDF membrane.
3. Block PVDF membrane in 5% skim milk in PBST at room temperature for one hour.
4. Apply hybridoma culture supernatant/ ascites/ purified Ig as primary antibody. Incubate the membrane at room temperature on an orbital shaker for one hour.
5. Wash 5 times with PBST.
6. Apply HRP conjugated secondary antibody, and incubate the membrane at room temperature on an orbital shaker for one hour.
7. Wash 5 times with PBST.
8. Use UVP autochemi/ ECL system for signal detection (to visualize the result).
- Between each step, plates were adequately washed using PBST.
- Secondary antibody dilution, 1:10,000.
Primary Antibody/Dilution:
- Poly sera @ 6000X
- Cultured Supernatant @ 2X
- Ascites @ 1000X
- Purified Ig @ 1ug/ml
Diluents:
- 5% skim milk in PBST