

VAP1/AOC3 western blot

1. Human cultured cell lysates (SW480, Caco2, HeLa, Panc1, BxPC3, SKBr3) were prepared from 50-70% confluent cultures.
2. 20µg total protein was loaded to each lane.
3. Blocking was in 5% skim milk in PBS, overnight in cold room
4. Primary antibody. 1:1000 antibody dilution in 5% skim milk. Incubation was 1.5 hour in room temperature.
5. Rinse in PBS+0.05% tween20, 3x10min
6. Secondary anti-goat HRP antibody, 45-60min in room temperature.
7. Rinse in PBS+0.05% tween20, 3x10min
8. Signal detection with chemiluminescent reagents. Exposure time on X-ray film was 30 sec-5 min.