1. βTC3 cells were crosslinked with DSP (0.5mM) for 30 minutes at 37°C.
2. Nuclear extracts were prepared and subjected to either an IgG or NfatC1 Immunoprecipitation for 3 hours at 4°C.
3. Immune complexes were washed with RIPA buffer, and eluted with 150mM DTT dissolved in RIPA buffer.
4. Eluates were resolved by 10% SDS-PAGE, transferred to PVDF memrane and subjected to immunoblot analysis.
5. Block: 5% Nonfat milk in PBS+Tween
6. Primary: NFATC2-rabbit antibody, 1:1000, Overnight, 4°C
7. Secondary: mouse anti-rabbit IgG HRP, 1:2000, 2 hours, room temperature.
8. ECL treatment and exposed to X-Ray film