1. CFBE cell lysate (60 µg) were subjected to SDS-PAGE and western blotted to Nitrocellulose membrane.
2. Incubated the membrane in 25 ml of blocking buffer (TBST with 5% w/v nonfat dry milk) for 1 hr at room temperature.
3. Washed three times for 5 min each with 15 ml of TBST.
4. Incubated the membrane with H00010645-M02 antibody (1:2000 dilution) in 10 ml primary antibody dilution buffer (TBST with 5% BSA) with gentle agitation overnight at 4°C.
5. Washed three times for 5 min each with 15 ml of TBST.
6. Incubated the membrane with the Goat Anti-Mouse IgG, H & L Chain Specific Peroxidase Conjugate (Cat No: 401253 Merckmillipore) 1:10000 diluted in 10 ml of blocking buffer with gentle agitation for 1 hr at room temperature.
7. Washed three times for 15 min each with 15 ml of TBST.
8. Incubated the membrane with appropriate amount of Luminata Crescendo Western HRP substrate (WBLUR0100 Merckmillipore) for 5 min at room temperature.
9. Drained the membrane of excess developing solution (without letting it to dry) wrapped in transparent plastic sheet and exposed the x-ray film for 1 min.