

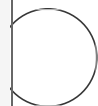


## sCD40L ELISA Assay

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### ABSTRACT

This protocol for the sCD40L ELISA kit (Cat#BMS6010, Invitrogen) is used to measure the concentration of sCD40L in serum and CSF samples in mice.

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**Protocol status:** Working  
We use this protocol and it's working

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- 1 Prepare Wash Buffer.
- 2 Wash the microwell strips twice with 300 µL Wash Buffer per well. Allow the Wash buffer to sit for 10-15s before harshly drop it in the strain. Wash again.
- 3 Empty wells on the paper towel, use the strip immediately after washing.
- 4 Prepare the standard dilution as the protocol. Using Sample Diluent (provided) to dilute samples.
- 5 Add 100 µl of Sample Diluent in duplicate to the blank wells.
- 6 For serum samples, add 50 µl of Sample Diluent to the sample wells and then add 50 µl of serum samples to the sample well. For CSF samples, add 5 µl of Sample Diluent to the sample wells and then add 95 µl of serum samples to the sample well.
- 7 Prepare Bio-Conjugate (fresh) and add 50 µl of Bio-Conjugate to all wells.
- 8 Apply an adhesive film to the plate and let it incubate at room temperature (RT) for 2 hrs on a microplate shaker.

- 9 Remove adhesive film and empty wells. Wash 4 times using Wash Buffer.
- 10 Prepare Streptavidin-HRP and add 100 µl of diluted Streptavidin-HRP to all wells.
- 11 Apply an adhesive film to the plate and let it incubate at RT for 1 hr on a microplate shaker.
- 12 Remove adhesive film and empty wells. Wash 4 times using Wash Buffer.
- 13 Add 100 µl TMB substrate solution to all wells. Incubate the strips at RT until the color changes. Avoid light.
- 14 Add 100 µl the stop solution to all wells.
- 15 Measure the absorbance of each microwell on a spectro-photometer at a wavelength of 450 nm.