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sCD40L ELISA Assay

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Fang Zhou[1,2]

[1]Massachusetts General Hospital; [2]Aligning Science Across Parkinson's (ASAP) Collaborative Research Network

Daniel Choo

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We use this protocol and it's working

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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.

Troubleshooting

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.