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Enzyme-linked immunosorbent assay (ELISA)

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We use this protocol and it's working

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Abstract

To analyze the expression of TNF- α, supernatants were collected at the end of each designated culture period, centrifuged and stored until subsequent enzyme-linked immunosorbent assay (ELISA). Total protein concentrations were determined by Bradford assay. The concentrations of TNF-α were measured using ELISA Quantitative Sandwich kits according to the manufacturer's instructions.

Materials

Bradford assay (5000006, Bio-Rad Laboratories, California, USA)
ELISA Quantitative Sandwich kits (MBS2701341, MyBiosource, San Diego, USA)

Troubleshooting

Safety warnings

The ELISA Quantitative Sandwich kit (MBS2701341) has high sensitivity and excellent specificity for detection of TNFa.

Before start

The ELISA Quantitative Sandwich kit (MBS2701341) was acquired from MyBiosource (San Diego, USA).

Sample preparation

1 Sample preparation and total protein concentration determination.

1.1 Cell culture supernatant was collected.

1.2 The supernatant was centrifuged 2000g x 10min x 4^{o}C. The samples were kept on ice.

1.3 Total protein concentrations were determined by Bradford assay (5000006, Bio-Rad Laboratories, California, USA) by measuring the absorvance (595 nm) with a plate reader (Varioskan LUX, Thermo Scientific, USA).

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2.1 The standard curve was prepared by serial diluting the stock standard solution in the diluent according to the manufacturer's instructions (MBS2701341, MyBiosource, San Diego, USA).

2.2 The standards, blank and samples were added into the pre-coated plate and incubate for 1h at 37^{o}C.

2.3 The liquid of each well was removed without wash. 100µL of Detection Reagent A working solution was added to each well and incubated for 1 hour at 37^{o}C.

2.4 The wells were washed with 1× Wash Solution three times. The remaining liquid from all wells was completely removed by snapping the plate onto absorbent paper.

2.5 100µL of Detection Reagent B working solution was added to each well and incubated for 30 minutes at 37^{o}C.

2.6 The wells were washed with 1× Wash Solution three times. The remaining liquid from all wells was completely removed by snapping the plate onto absorbent paper.

2.7 90µL of Substrate Solution was added to each well and incubated for 20 minutes at 37^{o}C.

2.8 Without removing or washing, 50µL of Stop Solution was added to each well.

2.9 The absorvance (450 nm) was measured immediately with a plate reader (Varioskan LUX, Thermo Scientific, USA). TNF-α levels were normalized to total protein concentrations.