

[SANDWICH] ELISA FOR DETECTING SECRETED

ICAM-1

by Chang-Duk Jun, 04/06/99

Purpose

Materials

- NUNC-Immuno Plate IIF.
- Horseradish Peroxidase-Streptavidin (Zymed; 43-4323)
- PBS or Balanced Salt Solution (BSS). Do not use RPMI or any medium that contains biotin.
- BSA (1% in PBS).
- ABTS substrate and substrate buffer (Zymed; #00-2011)
 - ABTS chromogen solution
 - Hydrogen Peroxidase solution
 - Citrate buffer
- Anti-ICAM-1 mAb R6.5 or CL203. Use as 10 ug/ml in PBS to coat plates.
- Biotinylated mAb: Biotin RR1/1, R6.1 and Biotin R6.5 supplied.
 - Use at a final concentration of 2 ug/ml (1:1000 dilution).
 - Do not refreeze. Stable at 4°C for weeks.
- Dilutions of sICAM-1 standards, 512 ng/ml -> 2 ng/ml, serial 2 fold dilutions in 1% BSA/PBS.
 - A. 512 ng/ml
 - B. 256 ng/ml
 - C. 128 ng/ml
 - D. 64 ng/ml
 - E. 32 ng/ml
 - F. 16 ng/ml
 - G. 8 ng/ml
 - H. 4 ng/ml
 - I. 2 ng/ml

Procedure

1. Diluate **mAb R6.5 (or CL203) to 10 ug/ml in PBS**. Add 50 ul diluted antibody to wells. Incubate 37°C for 1 h, or O/N at 4°C.
2. Wash 4X with PBS.
3. Block remaining protein-binding sites by adding **200 ul/well of 1% BSA in PBS**. Incubate 30 min at 37°C.
4. Wash 4X with PBS.
5. Add 50 ul of culture supernatant containing secreted ICAM-1. Include positive control wells of purified ICAM-1 ranging from 512 ng/ml to 2 ng/ml. Incubate 30 min at 37°C.
6. Wash 4X with PBS.
7. Add 50 ul/well **Biotinylated R6.5 diluted in 1% BSA/PBS** to a final concentration of 2 ug/ml (1:1000 dilution of stock). Incubate 30 min at 37°C.

8. Wash 4X with PBS.
9. Add 50 ul/well **HP-streptavidin** diluted according to manufacturers instructions. Current **dilution is 1:2000 in 1% BSA/PBS**. Incubate 30 min at 37°C.
10. Wash 4X with PBS.
11. Wash 1X with Substrate buffer without substrate.
12. Add 200 ul/well **Substrate buffer + ABTS Substrate**.
13. Incubate at room temperature until sufficient color develops.
May be as rapid as 2 min or as long as 20 min.
14. Read absorbance at 414 nm, blanking against negative control well.

Expected Results

We get our best curves when the maximum Abs. for the 512 ng/ml standard is < 1.500, but less than 1.800.