ELISA Measurement of Mouse IL-2

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[Abstract] Interleukin-2 (IL-2) is a cytokine secreted by T cells that is essential for immune system activation. This protocol is routinely used for quantification of IL-2 concentration in the supernant of cultured lymphocytes under various stimulations and co-culturing conditions. Following slight modification and optimization, this protocol can also be adapted to quantitatively measure other secreted proteins and bio-molecules.

Materials and Reagents

1. BD OptEIA ELISA set including IL-2 standard, capture antibody, detection antibody/enzyme reagent (BD Biosciences, catalog number: 555148)
2. Assay diluent (BD Biosciences, catalog number: 555213) or medium made of half RPM1640/10% FCS/PS and half RPM1640.
3. Substrate solution (BD Biosciences, catalog number: 555214)
4. NaCl
5. $NaHCO_3$
6. Na_2CO_3
7. Na_2HPO_4
8. KH_2PO_4
9. KCl
10. Tween-20
11. H_2SO_4
12. Coating buffer (see Recipes)
13. Wash buffer (PBST, pH 7.0) (see Recipes)
14. Stop solution (see Recipes)

Equipment

1. ELISA plates (100 plates/case) (BD Biosciences, catalog number: 353279) and plate sealers (100 plates/case) (PGC Scientifics, catalog number: 045-826)

2. 96-well plates for dilution (SARSTEDT AG, catalog number: 82.1583)
3. Multichannel pipette and pipette tips (eBay, catalog number: RT-L200F)
4. Reagent reservoir (50 ml, 5/bag) (Corning, Costar®, catalog number: 4870)
5. ELISA micro plate reader

Procedure

1. Prepare coating buffer. Dilute capture antibody in coating buffer (1:250 for lot #0000052895) and add 50 µl to each well. Seal plates and incubate overnight at 4 °C.
2. Wash 3 times.
3. Block: 200 µl Assay diuent to each well. Room temperature (RT) 1 h.
4. Wash 3 times.
5. Add 50 µl standard or sample to each well. RT 2 h.
   Standard: make 800 pg/ml standard in assay diluent from original 135 ng/ml stock and aliquote 0.4 ml each (enough for one assay) and store at -80 °C.
   135 ng/ml x 23.7 µl = 800 p/ml x (3.98+0.0237) ml

Pg/ml	0	50	100	200	400	800
800 pg/ml (µl)	0	9.38	18.8	37.5	75	150
Diluent (µl)	150	140.6	131.2	112.5	75	0

   50 µl each
6. Wash 5 times.
7. 15 min before use, dilute detection antibody and avidin-conjugated HRP in Assay diluent (1:330 for lot #0000052895, 8 µl each/6 ml for one 96-well plate). RT 1 h.
8. Wash 7 times.
9. Prepare substrate solution (1:1 of A and B, 6 ml for one 96-well plate). Add 50 µl to each well. RT 30 min in dark.
10. Add 25 µl stop solution. Read at 450 nm and 570 nm within 30 min.

Recipes

1. Coating buffer (0.1 M carbonate buffer) (pH 9.5)
   4.20 g $NaHCO_3$
   1.28 g Na_2CO_3 / 0.5 L
   Use within 7 days and store at 4 °C.
2. Wash buffer (PBST, pH 7.0)
   16 g NaCl
   2.32 g Na_2HPO_4

0.4 g KH_2PO_4

0.4 g KCl, 1 ml

Tween-20 / 2 L per two 96-well plates

Use within 3 days and store at 4 °C.

3. Stop solution

   2 N H_2SO_4 (1 M)

References

1. Huang, G. N., Huso, D. L., Bouyain, S., Tu, J., McCorkell, K. A., May, M. J., Zhu, Y., Lutz, M., Collins, S., Dehoff, M., Kang, S., Whartenby, K., Powell, J., Leahy, D. and Worley, P. F. (2008). NFAT binding and regulation of T cell activation by the cytoplasmic scaffolding Homer proteins. *Science* 319(5862): 476-481.